

# THE BOTANICAL GAZETTE

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WITH TWENTY-THREE PLATES AND FORTY-ONE FIGURES IN THE TEXT

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ERRATA.

P. 9, line 2 from below, for other read certain.

P. 10, line 13, for five read six.

P. 22, line 3, for 14 read 14.

P. 22, line 4, after 1887 insert ).

P. 182, line 14 from below; p. 183, line 16 from below; p. 185, line 14 from below; p. 186, line 5, for *Nothocalais* read *Nothocalais*.

P. 185, last line; p. 187, line 16, for Fost read Jost.

P. 213, line 11 from below, for Nawachsin read Nawaschin.

## BOTANICAL GAZETTE

JULY, 1900

ON THE ENDOSPERM AND EMBRYO OF PEPEROMIA  
PELLUCIDA.

DUNCAN S. JOHNSON.

(WITH PLATE I)

IN the spring of 1899 I began the study of the Piperaceæ, with well-preserved material of the genera *Peperomia*, *Heckeria*, and *Piper*, collected by the late Professor J. E. Humphrey on the unfortunate expedition to Jamaica in 1897. It was soon discovered that seeds were needed for sprouting in order to complete the work, and it was therefore laid aside temporarily and my time given to work on the related genus *Saururus*.

The recent paper by Professor Campbell ('99) on *Peperomia pellucida* announced results differing from those I had obtained, and led me to reexamine my slides, with the result that I was satisfied with the essential correctness of my former observations, and noted several interesting features in addition. As the intended detailed study of the group may be deferred for some time, an outline of the most important observations thus far made on *Peperomia pellucida* Kunth is given here.

The flower consists of two stamens, and a carpel sessile in the axil of a top-shaped bract (*br*, *fig. 1*). The ovule is single, basal, and orthotropous (*fig. 1*), with a single integument and one archesporial cell. The development of the flower as far as followed agrees with the account given by Schmitz ('72), and the development of the macrospore in the nucellus is as described by Campbell.

The primary archesporial cell cuts off a tapetal cell above, and then immediately forms the definitive macrospore below. The tapetum divides to three or four tiers of cells, which finally form, together with the outer layer of cells of the nucellus, a persistent plug of yellowish thick-walled cells, directly under the micropyle (*tp*, *figs. 1, 2, 9, 13*).

The first four nuclei formed from the large macrospore nucleus are located at the periphery of the pretty dense protoplast of the embryo-sac, arranged like the spores of a tetrad and connected by strands of granular cytoplasm (*es*, *fig. 2*). Soon after this we find eight ellipsoidal peripheral nuclei, imbedded in the cytoplasm surrounding the enlarged vacuole of the embryo-sac. Up to this stage the sequence of phenomena has not been very different from that in the normal angiosperm embryo-sac, except for the lack of the bipolar grouping usually found. But now each of the eight nuclei divides again, as Campbell has shown, to form an embryo-sac with sixteen similar nuclei, pretty uniformly distributed in the peripheral layer of cytoplasm (*fig. 1*). A little later than this, before the pollen tube reaches the embryo-sac, the cytoplasm begins to get denser about one of the nuclei at the top of the embryo-sac, and finally a definite limiting membrane surrounds this and forms the oosphere (*o*, *fig. 1*). This egg is not directly under the micropyle, but is pushed aside slightly by the aggregation of a smaller amount of cytoplasm about a second nucleus at the top of the embryo-sac to form the single *synergid*, as we may call it from its position (*sy*, *fig. 1*). The position of spindles in certain cases seems to indicate that this is a sister cell to the oosphere. Sometimes other nuclei are found near the egg, but often not, and in no case was there seen a definite massing of cytoplasm about any of these. At first the synergid does not have a definite wall, but later on, as it persists, at and after fertilization, a distinct wall can be seen (*sy*, *figs. 3, 7, 9, 11, 12*).

At about the time the pollen tube enters the egg certain of the remaining fourteen peripheral nuclei begin to move together to form a compact group, of usually eight nuclei, surrounded by

cytoplasm. This group may appear at the lower end of the embryo-sac, in the middle near the wall, or center, or above, and near or even in contact with the egg (*espn*, *fig. 3*).

The remaining peripheral nuclei retain their position near the wall, at first as naked nuclei in the thin layer of cytoplasm, but later each of these and a small portion of cytoplasm is separated from the great mass of cytoplasm in the embryo-sac by a flat saucer-shaped wall (*pn*, *figs. 3, 7, 9, 11, 13*). Nowhere was there noticed any tendency of a number of these to collect in a basal position, or anything in their behavior to suggest their homology with the antipodals of the typical angiosperm embryo-sac.

The short top-shaped stamens bear two pollen sacs each. Certain pollen grains (three or four in a section showing fifteen fertile ones) remain with unthickened walls, but apparently are not used for the nourishment of the fertile ones. The nucleus of the finely reticulate-walled pollen grain divides to two at a time soon after the formation of the tapetum in the embryo-sac of the same flower. The pollen grains are shed after the embryo-sac has reached the four-nucleate stage. They lodge on the large abaxial lobe of the carpel (*st*, *fig. 1*), and grow downward through a conical mass of small-celled conducting or nutritive tissue to the fusion canal of the carpel, and thence to the micropyle (*pt*, *fig. 1*). Just when the division of the generative nucleus occurs was not made out with certainty, but in cases where the pollen tube had just reached the embryo-sac the pollen-tube nucleus was seen at its very tip, and a single large generative nucleus, with cytoplasm about it, at the level of the nucellus. In other cases of about the same age there were apparently two generative nuclei.

No indication of a sterile prothallial cell was discovered. The pollen tube often extends some distance into the egg, and after its entrance two nearly similar nuclei are found within the egg. The exact fate of the pollen-tube nucleus and the second generative nucleus was not determined.

For a considerable time after its entrance the male nucleus lies in the egg near or even in contact with the female, but without

fusing with it (*figs. 3, 11*). Its presence in the egg seems, however, to have an important influence on the other contents of the embryo-sac. At the time of its entrance the group of eight nuclei, each with a single nucleolus, is usually found in the central or upper part of the embryo-sac near the egg, surrounded by a considerable mass of cytoplasm, but not separated from each other by cell walls (*espn, fig. 3*). Soon after this the walls of certain of these nuclei are seen to be flattened against each other (*espn, figs. 3, 6*), and a little later still the group may consist of one or two larger, elongated, constricted nuclei, each with two nucleoli, and six or four normal-sized nuclei each with a single nucleolus. These larger nuclei have been formed in each case by the fusion of two of the original nuclei of the group. The first one or two of these fusion nuclei form centers to which the other nuclei of the group draw up closely and fuse on to add their bulk to that of the larger nuclei. Finally a very large nucleus is formed, with at first several normal sized nucleoli, and later fewer very much larger ones or a single one (*espn, figs. 6, 7*). The wall of this large nucleus shows at first several projecting lumps or knobs, each indicating the portion contributed by one of the fusion nuclei (*espn, figs. 4, 5, 6, 7, 8*—a series of sections of the same group). The line of contact of the walls of the fusing nuclei is at first evident by the darkly stained region where the two peripheral chromatin nets press against each other (*espn, figs. 5, 6, 7*). Later the lumps on the wall gradually smooth out and the chromatin net becomes evenly distributed about the periphery of the usually transversely elongated nucleus, which lies in a pretty dense mass of cytoplasm just below the oospore (*espn, figs. 7, 8*).

From this time on this nucleus behaves like the endosperm nucleus of the typical angiosperm embryo-sac. It sometimes begins its development before any activity is noticed in the fertilized egg, except that the wall of the latter becomes more distinct and the sexual nuclei flatten against each other (*fig. 11*). In other cases the sexual nuclei fuse during the fusion of the endosperm-forming nuclei (*fig. 7*). The endosperm nucleus

divides by mitosis, the first spindle being approximately transverse. The number of chromosomes here is seemingly very large, compared with that in other spindles found in the embryo-sac or in the nucellus, but they were so densely packed in all the cases seen that it was impossible to make accurate counts.

It is certain, however, that the amount of chromatin in this and its daughter spindles is greater than that found anywhere else in the plant (*espn*, *figs.* 9, 11). A cell plate is formed in the typical way at the middle of each spindle, the fibers of the latter being stretched out laterally to a surprising extent (*espn*, *fig.* 10). The new cell-wall thus formed stretches from the oospore to the base of the embryo-sac and cuts the latter completely in two, forming thus two endosperm cells. Each of these divides further, forming a cell-wall immediately at each division, till in the oldest seeds seen there are forty or more endosperm cells, each with a large nucleus, several nucleoli, and dense cytoplasm, filling up all of the embryo-sac not occupied by the embryo and synergid and flattening the degenerating peripheral nuclei against the wall of the embryo-sac (*esp*, *figs.* 12, 13, 14, *pn*, *figs.* 9, 13).

The fusion of the sexual nuclei is completed, at the latest, soon after that of the nuclei of the endosperm group, and before many endosperm cells are formed the oospore divides to form the embryo. In the few cases of the early divisions of the embryo seen the first wall seemed to be longitudinal, and the position of the walls in the slightly older embryos, often seen, seemed to confirm this (*em*, *figs.* 12, 13).

The oldest fruits available, as was evident from their position on the spike were nearly ready to separate from the mother plant, *i. e.*, were nearly ripe. In these the embryo consisted of more than twenty cells, but showed no sign of a definite suspensor and no indication of the organs of the young sporophyte. In fact, the whole structure has much the same shape and but slightly larger size than the one-celled oospore (*osp*, *fig.* 9, *em*, *figs.* 12, 13).

The ultimate fate of the long persistent synergid has not been made out with certainty as yet, but so far as it has been definitely traced it retains much the same size and appearance that it has at the time of fertilization, except that the wall becomes more distinct (*sy*, *figs.* 3, 7, 9, 11, 12). In many cases where the embryo consisted of six or eight cells a single large cell could still be seen beside it, which seemed quite distinct from the endosperm cells that press against the embryo on all other sides, and this is interpreted as the still persistent synergid (*sy*, *fig.* 12), and possibly the cell at the right of the embryo under the tapetal plug in *fig.* 13 is another case of the same sort. In several of the very oldest embryos seen there was a group of cells, smaller than any other cells in the embryo-sac, located in in this same position beside the embryo. The evidence obtained points to these as derivatives of the synergid, but further careful work, including probably the study of the sprouting seed, will be necessary to make this certain and to determine the ultimate fate of these cells.

The absolute size of the embryo-sac in these mature seeds is but little larger than when the egg is differentiated (*figs.* 2, 3, 13—note the magnification of each). The relative size and position with reference to the other parts of the fruit is shown in *fig.* 14. The oblate spheroidal mass of endosperm is about one eighth the length of the whole seed. It is separated from the integument at the top by three or four layers of cells of the tapetum and nucellus. Below is the great mass of perisperm cells developed from the basal portion of the nucellus (*figs.* 12, 14). Each of these cells is finally packed closely with starch, aggregated in several masses (filling up the vacuoles) separated by thin layers of cytoplasm (*psp*, *figs.* 13, 14, 15), in one of which lies the flattened and distorted, but still darkly staining nucleus (*pspn*, *fig.* 15). In these cytoplasm layers are also found large clear, or finely granular, spherical masses of an undetermined chemical nature, but presumably serving as food (*psp*, *fig.* 15.)

The single integument is but two cells in thickness, and both of these take part in the formation of the seed coat or



testa. All the walls of the cells of the outer layer become thickened till the cell cavity is practically obliterated, and the thickness of this layer is about the same over the whole of the seed, or slightly greater toward the base (*int, figs. 14, 15*). The cells of the inner layer thicken the outer walls greatly, especially near the upper end of the seed, where large knobs of the thickening substance project into the cell cavity (*int, figs. 14, 15*). The cavity is never entirely filled as in the outer cells, but considerable space remains which is packed with starch like the cells of the perisperm (*int, figs. 14, 15*). The innermost layer of thickening substance of the outer walls of the cells of this layer is of quite different consistency from the rest of the wall, and shows in sections as a uniform border about all the hollows and projections of the latter.

At the base of the seed several layers of cells of the chalaza thicken their walls, like those of the outer integument layer, to complete the protection of the seed (*fig. 14*).

The seed does not escape from the carpel, but the latter apparently remains adhering closely to it when the whole falls from the mother plant. At this time the carpel is four or five layers thick, except at the base and in the stigmatic region (*cp, fig. 15*). The outer layer is of large, cuboidal, nearly empty cells, interspersed with knob-like hydathodes. Its cells have unthickened walls, except for the fine striae found quite generally on the outer epidermal walls of the whole inflorescence (*cp, fig. 15*). Next within this layer we find two or three layers of thin-walled flattened cells, with little contents. Closely adherent to the integument is the inner layer of the carpel, made up of large cells of about equal height and meridional length, but elongated equatorially to twice this length. These cells have the basal wall considerably thickened, with comparatively low ridges projecting above this general thickening (*fig. 15*). The lateral and outer walls of these cells have anastomosing ribs surrounding thin spots or pits, forming cells closely resembling those of the velamen of the roots of many epiphytes in structure, and perhaps in function also. The basal or inner end of these cells is

occupied by a granular mass, apparently of some firm substance deposited by the protoplast as an addition to the protective layers of the fruit and seed, or possibly connected with the absorption of water by these cells (*fig. 15*).

The subtending bract increases but little in size after the macrospore is formed, and as the fruit ripens the bract withers and is squashed down by the swelling carpel (*br. figs. 1, 14*).

In comparing the foregoing with Campbell's results it will be seen that my observations confirm his in regard to the origin of the macrospore and its development to a sixteen-nucleate ripe embryo-sac. Campbell thinks that one of the upper of these nuclei goes to the egg, and one to each of the two naked synergids; while eight others, which he interprets as probably antipodals, temporarily collect at the base of the embryo-sac, but later disperse and become indistinguishable. The other five nuclei play no prominent part, there being according to his observations no nuclear fusion analogous to that of the polar nuclei of the ordinary angiosperm embryo-sac.

In my own work I have seen but a single synergid which is long persistent and has a distinct wall. The nuclei of the group which Campbell interprets as possible antipodals I find are ultimately fused together into one endosperm nucleus, there being no special basal (antipodal) group of sterile cells or nuclei.

Again, Campbell says that the at first flattened embryo finally fills the whole embryo-sac and that there is no endosperm whatever, while I find that the embryo is nearly globular at first and is later completely surrounded by endosperm which fills the greater bulk of the embryo-sac.

The meaning of these very striking peculiarities of the embryo-sac of *Peperomia pellucida* (and other species of the same genus) is not easy to determine. The extra division of the embryo-sac is quite unique, and so also is the lack of a basal group of sterile cells or antipodals. Finally the fusion of so large a number of nuclei into one, in forming the endosperm nucleus, is approached only by the cases of fusion, at quite a

different stage of development, of the several nuclei in the endosperm cells of *Staphylea pinnata* and *Corydalis cava*. In these forms, according to Strasburger ('80), when walls appear about the endosperm nuclei several of these are enclosed in a single cell and these later fuse to a single nucleus. The fusion of polar nuclei during instead of before fertilization is found also in *Allium fistulosum* (Strasburger '79, p. 21), and this case may perhaps be considered as analogous to that of the endosperm-forming nuclei in *Peperomia*.

That these peculiarities of *Peperomia* are to be considered primitive rather than higher specializations seems to me unwarranted by the evidence at present available, especially when we consider the fact, which I have ascertained, that such closely related genera as *Piper*, *Heckeria*, and *Saururus* have essentially typical angiosperm embryo-sacs. These latter forms develop a small amount of endosperm in a manner similar to that found in such distantly related and certainly not very primitive forms as the *Nymphaeaceæ*.

Again, the lack of any grouping of the extra peripheral nuclei in the embryo-sac of *Peperomia* fails to give any encouragement from this source to those who look upon the antipodal group in the angiosperms as a second egg-apparatus (Lotsy, '99, p. 106).

So also the fusion of the eight nuclei to form the endosperm nucleus, if we regard it as at all homologous with that of the polar nuclei, seems to indicate that this is a purely vegetative or nutritive process, rather than anything like a sexual fusion as suggested by Mann ('92). Finally, the development of the cell walls in the endosperm directly after nuclear division each time, instead of by the method of free cell formation, as in the prothallus of the higher pteridophytes, is not favorable to the view that *Peperomia* is a transitional form between these forms and the typical angiosperms.

I am inclined to believe that the peculiarities of the embryo-sac of *Peperomia* have been secondarily acquired, and are analogous to those found in other angiosperms of peculiar habit, *e. g.*, many aquatic, parasitic, and saprophytic forms.

It is probable that a careful study of the sprouting seed will show the meaning of some of these peculiarities to the plant. I hope soon to be able to determine whether the tissue which I have called endosperm here has the same function as in *Saururus*, of absorbing the perisperm for the benefit of the embryo during the sprouting of the seed. I trust also that a further study of related forms may discover some intermediate type of embryo-sac, that will indicate more definitely the possible derivation of the peculiar one found in *Peperomia*.

In conclusion and summary: the macrospore nucleus of *Peperomia pellucida* forms sixteen free nuclei, of which one goes to the egg, one to the synergid, eight more fuse to form a single endosperm nucleus, while the other five remain sterile and degenerate. The nearly ripe seed contains an embryo of fifteen or more cells surrounded by endosperm cells in which the walls are formed directly from the cell plate of the spindle.

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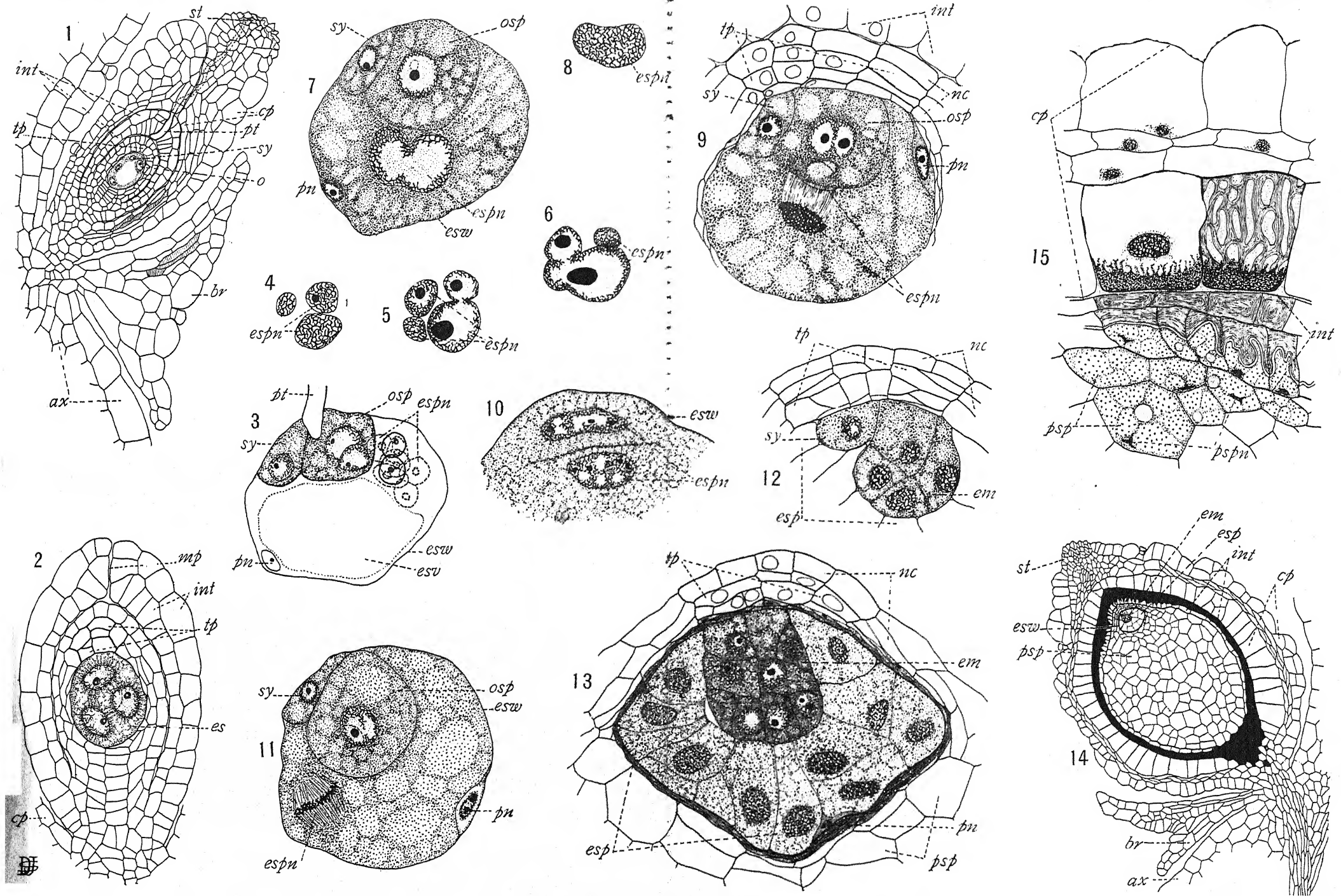
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#### EXPLANATION OF PLATE I.

Abbreviations used: *ax*, axis of the inflorescence; *br*, subtending bract; *cp*, carpel; *em*, embryo; *es*, embryo-sac; *esp*, endosperm; *espn*, endosperm nucleus; *esv*, principal vacuole of embryo-sac; *esw*, wall of embryo-sac; *int*, integument; *mp*, micropyle; *nc*, nucellus; *o*, oosphere nucleus; *osp*, oospore;





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*pn*, peripheral nucleus of the embryo-sac; *psp*, perisperm; *pspn*, nucleus of perisperm cell; *pt*, pollen tube; *st*, stigmatic lobe of carpel; *sy*, synergid; *tp*, tapetal cells.

All figures are camera drawings from microtome sections.

FIG. 1. Longitudinal section of axis, bract, and carpel containing nearly ripe (sixteen-nucleate) embryo-sac.  $\times 150$ .

FIG. 2. Longitudinal section of an ovule with a four-nucleate embryo-sac.  $\times 440$ .

FIG. 3. Longitudinal section of an embryo-sac after the entrance of the pollen tube and male nucleus into the egg, and showing the group of nuclei that fuse to form the single endosperm nucleus (those with dotted outlines are in the next section to the one from which the rest of the figure is drawn).  $\times 775$ .

FIGS. 4-8. A series of sections of a group of nuclei fusing to form the endosperm nucleus, in an advanced stage of fusion and with large fused nucleoli; in *fig. 7* the other contents of the embryo-sac are shown; the figures are numbered in the order of succession of the sections.  $\times 775$ .

FIG. 9. Longitudinal section of embryo-sac, showing the fusion of male and female nuclei in the egg, and a spindle of the second division of the endosperm nucleus; the upper nucleus of spindle from another section.  $\times 775$ .

FIG. 10. Part of a tangential section of embryo-sac, showing beginning of the formation of a cell-wall from the cell plate of the dividing endosperm nucleus.  $\times 775$ .

FIG. 11. Approximately transverse section of the upper end of an embryo-sac like that shown in *fig. 9*.  $\times 775$ .

FIG. 12. Longitudinal section of the upper end of an embryo-sac through the synergid and an eight-celled embryo.  $\times 775$ .

FIG. 13. Longitudinal section through the upper end of nucellus of a nearly ripe seed, containing an embryo of twenty or more cells surrounded by endosperm.  $\times 775$ .

FIG. 14. Longitudinal section through axis, bract, and a nearly ripe fruit; endosperm and embryo practically as in *fig. 13*.  $\times 75$ .

FIG. 15. Part of section of carpel, integument, and perisperm from *fig. 14* (at the left from the base of the embryo-sac).  $\times 775$ .



## NEW OR UNRECORDED MOSSES OF NORTH AMERICA. I.

J. CARDOT and I. THÉRIOT.<sup>1</sup>

(WITH PLATES II-V)

*PHASCUM CUSPIDATUM* Schreb. var. *Americanum* Ren. & Card., var. nova.—Costa longe excurrente apice saepius decolorata varietati *piliferum* proximum, sed foliis brevioribus, superne magis papillois pedicelloque brevissimo erecto distinctum. Varietas *mitraeforme* Limpr., foliis papillois similis, differt foliis majoribus longioribusque, costa minus longe excurrente et calyptra conico-mitraeformi.

Wisconsin: Madison, on ground in pastures, clover fields, and fallow ground (L. S. Cheney, 1893. Ren. & Card., *Musci Amer. sept. exsicc.*, no. 267). Missouri: old fields near Emma (C. H. Demetrio, 1891). Illinois: —

*Microbryum Floerkeanum* var. *Henrici* Ren. & Card. in BOT. GAZ. 14:91. 1899, from Kansas, leg. Henry, seems to be also a stunted form of the same moss. It has also the calyptra cucullate, a character which separates it from *Microbryum Floerkeanum*.

All the specimens we have received from North America as *Phascum cuspidatum* belong to this var. *Americanum*.

*GYMNOSTOMUM CURVIROSTRE* Hedw. var. *COMMUTATUM* Card. & Thér. (*Hymenostylum commutatum* Mitt., *Musci Ind. Or.*, p. 32. *Weisia curvirostris* var. *commutata* Dicks., *Handb. Brit. Mosses*, 212).

Newfoundland (Rev. A. C. Wagborne).

This variety has long, narrow leaves, and the cells of the areolation are everywhere long and smooth. In the type, the upper areolation is composed of irregular cells, rectangular, quadrate, and triangular, with scattered papillae.

*GYMNOSTOMUM CURVIROSTRE* Hedw. var. *SCABRUM* Lindb., *Musci Scand.* 22.

<sup>1</sup> Besides the new species, we shall describe in this paper and the following several species named by Renauld and Cardot in the *Revue Bryologique*, 1892-1893, but of which only short, provisional diagnoses were published.

Missouri: Benton county, on moist rocks along Indian creek (C. H. Demetrio, 1893). Minnesota: Lewiston cave (J. M. Holzinger, 1889), Bear creek (J. M. Holzinger, 1890). Wisconsin: Madison (L. S. Cheney, 1892. Ren. & Card. *Musci Am. sept. exsicc.* no. 269).

This form shows contrary variations to the preceding: the leaves are smaller, the cells quadrate, papillose; besides, the stem and the nerve are generally covered with high papillae.

HYMENOSTOMUM MICROSTOMUM R. Brown, Trans. Linn. Soc. 12: 572 (*Gymnostomum microstomum* Hedw., *Musc. frond.* 3: 71, *pl.* 30 B).

According to Lesquereux and James, *Manual* 56, this species is not known from North America, and all the specimens that have been communicated under the generic name *Hymenostomum* are to be referred to the *Weisia viridula* var. *gymnostomoides* C. Müll. Yet the no. 54 of Sullivan and Lesquereux *Musci Bor. Am.* belongs undoubtedly to the *Hymenostomum microstomum* R. Br., at least in our set.

Quite inseparable from *Weisia viridula* as to the vegetative organs, and differing only by the capsule closed with a membrane finally perforated in the center.

*Weisia Wimmeriana* BS., *Bryol. Eu.* 33-36: 4, *pl.* 1. (*Gymnostomum Wimmerianum* Sendtn. in *Flora* 23: 59. 1840. *Hymenostomum murale* Spr. *Musc. Pyr.* no. 236. *Gymnostomum murale* Sch. *Syn.* 37. 1860. [Ed. 1.] )

Minnesota: Taylor's Falls (J. M. Holzinger, 1895).

Resembling the slender forms of *W. viridula* in size, habit, shape, and areolation of the leaves; distinct chiefly by the inflorescence, which is parious, or sometimes, as in our Minnesota specimens, synoious. The peristome is generally rudimentary; in our American specimens, however, the teeth are rather perfect, with 4-5 articulations.

DICHODONTIUM OLYMPICUM Ren. & Card., *Rev. Bryol.* 19: 74. 1892.—Dioicum, humile, caespitosum, obscure viride. Caulis gracilis, erectus, 5-7<sup>mm</sup> altus. Folia madida patentia subrecurva, sicca laxe erecto-flexuosa, apice incurvata, 1.50-1.75<sup>mm</sup> longa, 0.6<sup>mm</sup> lata, e basi ovata vel oblonga paulo latiore breviter linguata, obtusa subobtusave, subundulata, marginibus plana, cellulis prominulis minute crenulata, supra basin integram distincte denticulata, costa valida percurrente vel subpercurrente; cellulae

minutae, obscurae, parietibus incrassatis, irregulariter quadratae,  $5-8\mu$  metientes, utraque pagina papillis prominentibus ornatae, cellulae inferiores majores, elongate rectangulares, laeves. Folia perichaetialia longiora, basi laxius reticulata, superne papillis elongatis subcylindricis obsita. Capsula in pedicello pallido,  $6-7\text{mm}$  longo, siccitate dextrorsum torto erecta inclinatave, oblonga, arcuatula, circa  $1.5\text{mm}$  longa,  $0.5\text{mm}$  crassa, collo distincte strumoso, sicca plicatula et sub ore leniter constricta; operculum ignotum. Peristomium purpureum, elatum, dentibus triangulari-lanceolatis, 15-20-articulatis, usque ad medium in 2-3 crura subulata fissis, longitudinaliter striolatis minuteque granulosis. Planta mascula ignota. *Plate II.*

Washington: Olympic mountains (L. F. Henderson).

A true miniature of *D. pellucidum* Sch., from which it is easily distinguished by the much smaller size, the leaves denticulate above the base, the cells of the areolation much smaller and with more prominent papillae and the strumose neck of the capsule.

DICRANELLA LAXIRETIS Ren. & Card., Rev. Bryol. 20: 30. 1893.—Dioica, pusilla, gregaria. Caulis simplex, brevissimus,  $1-2\text{mm}$  altus. Folia madida mollia, erecto-patentia, sicca flexuosa subcrispata, ascendendo sensim majora, circa  $2\text{mm}$  longa,  $0.25-0.3\text{mm}$  lata, lineari-lanceolata, apice obtuso minute denticulato, marginibus integris planis vel parce reflexis, costa percurrente vel sub summo apice evanida, rete laxo, cellulis inferioribus majoribus elongate rectangulis, superioribus breviter rectangulis subquadratisve, omnibus parietibus angustis. Folia perichaetialia vix diversa. Capsula in pedicello tenui pallido,  $5-6\text{mm}$  longo, erecta, minuta, oblongo-subcylindrica, arcuatula,  $0.75-1\text{mm}$  longa, operculo convexo oblique longe subulato. Annulus e duplici vel triplici serie cellularum compositus. Peristomii dentes purpurei, valde trabeculati, longitudinaliter striolati, usque ad  $\frac{2}{3}$  inferiora in 2 crura longa subulata bifidi. Planta mascula ignota. *Plate III.*

Louisiana: in a deep and shaded ravine near Lafayette (A. B. Langlois, 1891).

One of the smallest species, resembling *D. debilis* L. & J., but with shorter stems, softer and more flexuous leaves, which are minutely denticulate

at apex, softer areolation of wider, shorter, and thin-walled cells, and narrower rather asymmetrical capsule.

DICRANELLA HOWEI Ren. & Card., Rev. Bryol. 20: 30. 1893, et in Bull. de l'Herb. Boissier 4: 15. 1896.—Dioica, caespitosa, subnitens, lutescenti-viridis. Caulis erectus, simplex vel parcissime divisus, 6–10<sup>mm</sup> altus. Folia laxiuscula sicca erecto-flexuosa, madida plerumque plus minus subsecunda, 1.5–2.2<sup>mm</sup> longa, 0.28–0.35<sup>mm</sup> basi lata, lanceolato-subulata, acuta subacutave, integerrima vel summo apice obsolete denticulata, marginibus ubique planis, costa lata,  $\frac{1}{4}$ – $\frac{1}{3}$  basis et totam fere subulam occupante, cellulis angustis, linearibus, inferioribus brevioribus. Folia perichaetia e basi subvaginante longius et tenuius subulata. Capsula in pedicello rubello, siccitate sinistrorsum torto, 5–7<sup>mm</sup> longo, subhorizontalis, oblonga, arcuata, circa 1<sup>mm</sup> longa et 0.28–0.35<sup>mm</sup> crassa, sicca sub ore valde contracta, operculo alte conico. Annulus nullus. Peristomium *D. variae*.—Plate III.

California: Mt. Tamalpais, Marin county, on wet banks (Marshall A. Howe, 1892–1893. Ren. & Card., *Musci Amer. sept. exsicc.* no 203).

Very closely allied to *D. varia* Sch., of which it may be only a subspecies or a regional race; characterized by the more distant and more flexuous leaves, subsecund when moist, generally longer, plane on the borders, the broader nerve, the narrower cells, the somewhat narrower capsule in dry state, and the green-yellowish and brighter tinge of the tufts.

DICRANUM VIRIDE BS. var. *laeve* Ren. & Card., var. nova.—A forma typica habitu multo laxiore foliisque minus congestis dorso laevibus distincta.

Newfoundland: Bay-of-islands, old stump (Rev. A. C. Waghorne, 1895).

DICRANUM ANGUSTUM Lindb., Soc. pro Fauna et Fl. fenn. 1880, et Rev. Bryol. 9: 83. 1882. Lindb. & Arn., Musc. As. Bor. 2: 80 (descriptio locupletissima).

Northwest shore of Hudson Bay, lat. N. 63°55', long. O. 90°20' (G. Comes, 1893–1894). We found some stems of this rare species amongst specimens of *Aulacomnium turgidum*.

A polar moss, known only from some localities of north Finland and from Siberia. It is easily distinguished from *D. Bonjeani* De Not. (*D.*

*palustre* BS.) by the leaves straight, not undulate, convolute, and entire, the thinner costa, the less porose cells, and the perichaetial leaves long piliferous.

*FISSIDENS BRYOIDES* Hedw., var. *GYMNANDRUS* Ruthe, Hedw. *g*: 178. 1870. *Limpr.* Laubm. 1: 430. (*F. gymnandrus* Buse *Musc. neerl. exsicc. fasc. 4, no. 77*).

Northwestern Montana: in the vicinity of Lake MacDonald, Flathead county (J. M. Holzinger and J. B. Blake, 1898).

A peculiar form, easily distinguished from the type by the antheridia naked in the axils of the stem-leaves.

*FISSIDENS SUBBASILARIS* Hedw. var. *Bushii* Card. & Thér., var. nova.—A forma typica differt foliis latioribus brevioribusque, obtuso rotundatis, nervo fere ad apicem producto et areolatione magis opaca cellulis parietibus crassioribus.

Missouri: Eagle rock, on gravelly ground (B. F. Bush, 1897).

*Desmatodon systilioides* Ren. & Card., sp. nova.—Monoicus, gregarius. Caulis brevis, erectus, 2–3<sup>mm</sup> altus. Folia in rosulam congesta, patula, oblongo-lanceolata, apice sat subito breviterque acuminata, acuta, marginibus planis superne inaequaliter denticulatis, nervo valido rufescente percurrente vel breviter excedente, cellulis inferioribus laxissimis, subrectangulis, inanis, hyalinis vel lutescentibus, mediis et superioribus minutis, rotundato-subquadratis vel subhexagonis, papillis numerosis obscuratis, marginalibus 2–4-seriatis, quadratis vel breviter rectangulis, vix vel parum papillois, limbum distinctum lutescentem translucentem efformantibus. Folia perichaetalia vix diversa, paulo breviora. Capsula in pedicello lutescente vel pallide rubente, siccitate dextrorsum torto, 8–12<sup>mm</sup> longo erecta, breviter oblonga, brunnea, operculo oblique breviterque conico, subrostrato, apici columellae diu adhaerente, annulo peristomioque, ut videtur, nullis. Sporae laeves, 18–20 $\mu$  crassae.—*Plate II.*

Labrador: l'Anse-au-Mort (Rev. A. C. Waghorne, 1894).

By the lid attached to the columella and persistent after the opening of the capsule this species resembles *D. systilius* BS., but is clearly distinct from it by the leaves of a more obscure and more papillose areolation, with a distinct pellucid yellowish border, the nerve stouter, brownish, not or very

shortly excurrent, and by the total lack of peristome; at least, all the capsules we have been able to examine do not show the slightest trace of this organ. The pellucid border of the leaves shows some relationship between *D. systylioides* Ren. & Card. and *D. Porteri* James, but the latter has a much narrower capsule with a highly conic lid not adhering to the columella, and the peristome and annulus are well developed.

*Barbula eustegia* Card. & Thér., sp. nova.—Dioica? gregarie caespitosa. Caulis brevissimus, 1–2<sup>mm</sup> altus. Folia siccitate erecto-flexuosa, madida recurvo-patula, lineari-lanceolata, acuta, subacuta obtusiusculave, superne plicato-canaliculata, inferiora minima, 0.5<sup>mm</sup> longa, superne sensim majora, superiora 1<sup>mm</sup> longa, marginibus planis vel parce revolutis, integris, costa sub apice evanida vel eum fere attingente, dorso papillosa, cellulis inferioribus pellucidis, laxiusculis, laevibus, oblongis, subrectangularibus, sequentibus quadratis, superioribus parvis, vix 4–5 $\mu$  metientibus, quadrato-rotundatis, obscuris, minute papillosis. Folia perichaetialia caulinis majora, externa e basi semivaginantem sat subito in acumen elongatum canaliculatum, patulo-arcuatum constricta, interna oblonga, late breviterque acuminata, rete omnino pellucido. Capsula in pedicello capillari pallido, 12–18<sup>mm</sup> longo, siccitate dextrorsum torto, erecta vel obliqua, oblonga, 1–1.2<sup>mm</sup> longa, 0.3–0.4<sup>mm</sup> crassa, operculo conico-subulato capsulam aequante vel superante. Annulus duplex, 0.07<sup>mm</sup> latus. Peristomium purpureum, membrana basilari 0.08<sup>mm</sup> alta, dentibus circa 1<sup>mm</sup> longis bis convolutis, valde granulosus. Sporae laeves, 8 $\mu$  crassae. Flores masculi ignoti. Verisimiliter dioica.—*Plate IV.*

Idaho: Cedar creek, Latah county, on ground (L. F. Henderson, 1897).

This moss, received from Mr. Henderson only in very small quantity, seems a miniature of *B. flavipes* BS., from which, besides in its small size, it differs chiefly by the lid as long as the capsule or even longer. By this character, as well as by the form of the leaves, it resembles also *Trichostomum dicranoides* Sch. (*T. macrostegium* Sull. Icon. Suppl. 35, pl. 22) from Central and South America and the Antilles, which has been also recorded from Alabama; but this last species has the beak of the lid thinner, the leaves larger, broader, denticulate above, a less opaque areolation of larger and more distinct cells, and the peristome less twisted, with a shorter basilar membrane.

*Grimmia pseudo-montana* Card. & Thér., sp. nova.—Dioica, obscure viridis, densiuscule pulvinata. Caulis erectus, dichotome ramosus, circa 1<sup>cm</sup> altus. Folia sicca et madida erecta, subimbricata 1.75–3<sup>mm</sup> longa, 0.75–1 lata, ovato-lanceolata, marginibus planis integerrimis, inferiora mutica, superiora pilo breviusculo minute denticulato, basi paulo decurrente instructa, costa sat valida, basi 60–80 $\mu$  lata, superne canaliculata, apicem versus indistincta, cellulis inferioribus juxta costam linearibus, lutescentibus, margines versus quadratis vel breviter rectangulis, hyalinis, caeteris parvis, obscuris, quadratis vel subrotundatis, bistratosi. Folia perichaetialia subsimilia, basi laxius reticulata. Calyptra cucullata. Capsula in pedicello crassiusculo stricto, 1.5–2.5<sup>mm</sup> longo, exserta, erecta, breviter oblonga, sicca subcylindrica, exannulata, operculo convexo-rostrato. Peristomii dentes flammei, circiter 0.35<sup>mm</sup> alti, integri vel parcissime perforati, e basi late triangulari longe subulati, superne minute granulosi, articulis 15–25.—*Plate IV.*

Idaho: near Moscow, on dry rocks (L. F. Henderson, 1894).

Closely allied to *G. montana* BS., but sufficiently distinct by the larger leaves, with a stouter nerve and a shorter and a thicker hair, and chiefly by the peristomial teeth almost entire, not divided and scarcely perforated, with more numerous articulations.

GRIMMIA MONTANA BS. var. *Idahensis* Ren. & Card., var. nova.—A forma typica differt capsula pro more majore magisque exserta et pedicello paulo longiore, siccitate plerumque flexuoso subgeniculato. Folia pilo saepe destituta.

North Idaho: west end of Lake Pend d'Oreille (J. B. Leiberg, 1892. Ren. & Card., *Musci Amer. sept. exsicc.* no. 289).

GRIMMIA SUBSULCATA Limpr., Laubm. 1: 757.

Idaho (J. B. Leiberg, 1889; J. H. Sandberg, 1892). Northwestern Montana: in the vicinity of Lake MacDonald, Flathead county (J. M. Holzinger and J. B. Blake, 1898).

A long time confused with *G. alpestris* Schleich. The distinctive characters quoted by Limpricht are: for *G. alpestris*, pedicel straight, capsule without stomata, leaves not plicate; and for *G. subsulcata*, pedicel somewhat curved, capsule with stomata, leaves with two longitudinal folds in the upper

part. The last character is the best, for the pedicel of *G. subsulcata* is sometimes nearly straight and the capsule without stomata, while the folds of the leaves are always distinct, especially on a transverse section.

It is to be noticed that Limpricht cites erroneously *G. lamellosa* C. Müll. as a synonym for *G. alpestris*: on the contrary, from an original specimen Müller's plant is proved identical with *G. subsulcata*.

The true *G. alpestris* Schleich. has been gathered by Messrs. J. M. Holzinger and J. B. Blake in the same region of northwestern Montana where they have collected *G. subsulcata*.

**Orthotrichum Idahense** Card. & Thér., sp. nova.—Monoicum, laxe depresso-pulvinatum, inferne fuscum, superne lutescenti-viride. Caulis basi decumbens, longe denudatus, irregulariter ramosus, 2–3<sup>cm</sup> longus, ramis ascendentibus. Folia madida erecto-patentia, sicca erecto-appressa, 1.75–2.50<sup>mm</sup> longa, 0.50–0.75 lata, oblongo-lanceolata, subobtusa, integra, marginibus fere c basi usque infra apicem arcte lateque revolutis, costa subpercurrente; cellulae ubique unistratosae, parietibus valde incrassatis, inferiores rectangulares, juxta costam lineares, margines versus quadratae vel breviter oblongae, caeterae rotundae, utraque pagina papillis grossis bi-trifurcatis obsitae. Folia perichaetialia subconformia, basi laxius reticulata. Vaginula nuda. Capsula in pedicello brevissimo vix emersa vel semi-emersa, madida ovata, collo brevi attenuata, sicca subcylindrica, infra os leniter constricta, octostriata, striis e cellulis 4-seriatis, longioribus, lutescentibus, parietibus crassioribus compositis, stomatibus emersis. Operculum ignotum. Peristomii dentes 8 bigeminati, vel 16, siccitate reflexi, plus minus pertusi, minute granulosi, superne lineolati; cilia nulla vel fugacia. Calyptra lutescens, apice fusca, ramentis longis, numerosis, denticulatis, papillosis obsita. Sporae papillosae, 20–22<sup>μ</sup> crassae. Flores masculi sessiles.—*Plate V.*

Idaho: Moscow mountains, on rocks (L. F. Henderson, 1893).

By the superficial stomata and the peristome reflexed when dry and finely papillose this species belongs to the group of *O. arcticum* Sch., but is easily distinguished from all the other species of this group by its lax tufts, emergent capsule, and peristomial teeth less opaque, covered with less dense papillae.



ORTHOTRICHUM LYELLII H. & T. var. *Howei* Ren. & Card., var. nova.—A forma typica differt capsula exserta in pedicello eam aequante foliisque usque medium versus revolutis. Ab *O. papilloso* foliis minus flexuosis, siccitate strictulis, longius revolutis, acumine brevior et minus angusto distincta. Calyptra valde pilosa. Papillae foliorum parum prominentes. Folia nonnullis propagulis saepe instructa.

California: region of the upper Sacramento, Sisson, on trunks of *Quercus Kelloggii* (Marshall A. Howe, 1894. Ren. & Card. *Musci Amer. sept. exsicc.* no. 291).

WEBERA CARINATA Limpr., Laubm. 2: 261. (*Bryum carinatum* Boul., Musc. de la France 280. *B. naviculare* Card., Rev. Bryol. 13: 27. 1886, et *B. cymbuliforme* Card., loc. cit. 14: 22. 1887. *Webera cucullata* var. *carinata* Husn., Muscol. Gall. 229).

Northwestern Montana: in the vicinity of Lake MacDonald, Flathead county (J. M. Holzinger and J. B. Blake, 1898).

By the habit and the dioicous inflorescence, this moss approaches *W. commutata* Sch., from which it differs in the more slender stems, the leaves always plane on the borders, distinctly carinate on the back, imbricated in five rows, and the cell walls thinner.

*Bryum euryloma* Card. & Thér., sp. nova.—Dioicum, dense caespitosum, lurido-viride. Caulis erectus, tomentosus, 2–3<sup>cm</sup> altus. Folia conferta, madida erecto-patentia, sicca subappressa, e basi decurrente anguste lanceolata, 3–4<sup>mm</sup> longa, 0.65–0.75<sup>mm</sup> lata, sensim et tenuiter acuminata, integra vel apice obsolete denticulata, marginibus anguste revoluta, interdum uno latere subplana, nervo in cuspidem breviusculam, acutissimam, apice saepe decoloratam, subintegram vel denticulatam excurrente. Areolatio *B. pseudotriquetri*, limbo autem latiore e 5–6 seriebus cellularum composito. Folia perichaetia intima minora, triangulari-lanceolata, sensim cuspidata, margine plana. Capsula in pedicello 15–20<sup>mm</sup> longo basi atropurpureo, abrupte pendula, angusta, subcylindrica, sicca sub ore constricta, collo longe attenuato in pedicello sensim defluente instructa, 2.50–4<sup>mm</sup> longa, 0.75–1 crassa, operculo convexo apiculato. Peristomium *B. pseudotriquetri*. Flores masculi capituliformes.—Plate V.

Puget sound, Orcas island, Mt. Constitution, lake border (L. F. Henderson, 1892).

Distinct from *B. pseudotriquetrum* Schw. and allied species by the smaller size, the narrower leaves entire or scarcely denticulate at apex with broader margin, and the capsule smaller, narrower, and more abruptly pendulous.

*BRYUM CRASSIRAMEUM* Ren. & Card. var. *Covillei* Ren. & Card., var. nova.—A forma typica differt cespitibus densioribus, caulibus ramisque gracilioribus, strictioribus, foliis strictis magis appressis, costa pro folii magnitudine plerumque crassiore, capsula fusco-rubra et peristomii interni segmentis dorso latius apertis.

Rocky mountains (Death valley Expedition, no. 1358; F. V. Coville and F. Funston, 1891).

*BRYUM TORQUESCENS* BS., Bryol. Eur. 6-9: 49, *pl.* 20.

Washington: Pullman, Whitman county, moist banks (L. F. Henderson, 1892).

Nearly allied to *B. capillare* L., but distinct by the synoicous inflorescence and the capsule deep red when mature. The American form differs from the European type by the leaves being erecto-patent and not spirally contorted in dry state.

*PTEROGONIUM GRACILE* Sw. var. *Californicum* Ren. & Card., var. nova.—A forma typica Europaea differt foliis longioribus longiusque acuminatis cellulisque alaribus minoribus.

California: "ad rupes Californiae, perfrequens; Bolander" (Sulliv. et Lesq. *Musci bor. amer. exsicc.*, ed. 2, no. 349); Sansalito (Marshall A. Howe, 1892); Coast Range mts., San Mateo county, on trees (Marshall A. Howe, 1895; Ren. and Card., *Musci Amer. sept. exsicc.* no. 316). All the Californian specimens of *P. gracile* that we have examined belong to this variety.

*PYLAISIA POLYANTHA* Sch. var. *drepanioides* Ren. & Card., var. nova.—Forma peculiaris, habitu et magnitudine *Hypno pallescens* similis; folia secunda plerumque ad basin acuminis obsolete denticulata, cellulis alaribus minus numerosis et minus obscuris, rete magis scarioso. Capsula minor. Peristomium normale.

Minnesota: without locality or name of collector, mixed with a small form of *Hypnum uncinatum* Hedw. (Herb. Univ. of Wisconsin).

PSEUDOLESKEA PATENS Limpr. Laubm. 2:806. (*Leskea?* *patens* Lindb. in Soc. pro Fauna et Fl. fenn. 1880. *Lesquerenxia patens* Lindb. in Meddel. af Soc. pro Fauna et Fl. fenn. 14:75. 1887.

Newfoundland: Deer lake (Rev. A. C. Waghorne).

This species differs from *P. atrovirens* in its more slender stems, the leaves erecto-patent (not secund), symmetric (not falcate), and the papillae being set on the middle of the cells and not on the angles.

TRIPTEROCLADIUM LEUCOCLADULUM (C. Müll.) Jaeg. var. **camptocarpum** Card. & Thér., var. nova.—A forma typica differt tantum capsula brevi, subhorizontali, arcuata, brachythecioidea.

Idaho: Latah county (L. F. Henderson, 1894).

AMBLYSTEGIUM SERPENS Br. Eur. var. **subenerve** Ren. & Card., var. nova.—A caeteris formis minoribus *A. serpentis* differt foliis enervibus vel subenervibus. Ab *A. subtili* habitu robustiore, foliis multo majoribus latioribusque, brevius acuminate distinctum.

Newfoundland: Bay-of-islands (Rev. A. C. Waghorne).

AMBLYSTEGIUM FLUVIATILE Br. Eur. var. **brevifolium** Ren. & Card., var. nova.—A forma typica Europaea caule magis regulariter pinnato foliisque minoribus, brevioribus, ovato-acuminatis, costa pro folii magnitudine crassiore distinctum.

Minnesota: Lanesboro (J. M. Holzinger, 1894. Ren. and Card. *Musci Amer. sept. exsicc.* no. 327).

AMBLYSTEGIUM RIPARIUM Br. Eur. var. **longinerve** Card. & Thér., var. nova.—A forma typica nervo in acumen longius producto distinctum.

Arkansas: Varner, in water (B. F. Bush, 1898).

Resembles *A. vacillans* Sull. in the long-nerved leaves, but in this species the branch leaves have a short obtuse acumen, while in our moss they are narrowly and acutely acuminate, like the stem leaves.

HYPNUM HALLERI Linn. fl. apud Swartz Meth. Musc. 34.

Labrador: l'Anse-au-Mort (Waghorne, 1894); Cook's brook (Waghorne, 1897). Newfoundland: Middle Arm, on rocks (Waghorne, 1896).

A very distinct species of the subgenus *Campylium*, at once characterized by the very dense tufts, the stems entirely prostrate and divided into pinnate branches, the leaves much crowded, recurved-squarrose from a more erect base, minutely denticulate all around, and with a much shorter point than in the allied species.

HYPNUM CUPRESSIFORME L. var. RESUPINATUM Sch. Coroll. 133.  
(*H. resupinatum* Wils., Bryol. Brit. 398).

Newfoundland: Chance cove (Rev. A. C. Wagborne, 1891).

This variety, considered by many authors as a distinct species, is characterized by the leaves not falcate-secund, imbricate or homomallous and pointing upward, and the capsule erect and symmetrical or very slightly curved or inclined. It is connected with the type by intermediate forms.

HYPNUM MOLLE Dicks. var. SCHIMPERIANUM Sch., Syn. 775  
[ed. 2]. (*H. Schimperianum* Lorentz, Moost. 123, pl. 5, fig. c).

Northwestern Montana: in the vicinity of Lake MacDonald, Flathead county (J. M. Holzinger and J. B. Blake, 1898).

Differs from the type by the longer and more slender stems, naked below, and by the leaves smaller and with a shorter acumen.

STENAY and LE HÂVRE, FRANCE.

#### EXPLANATION OF PLATES II-V.

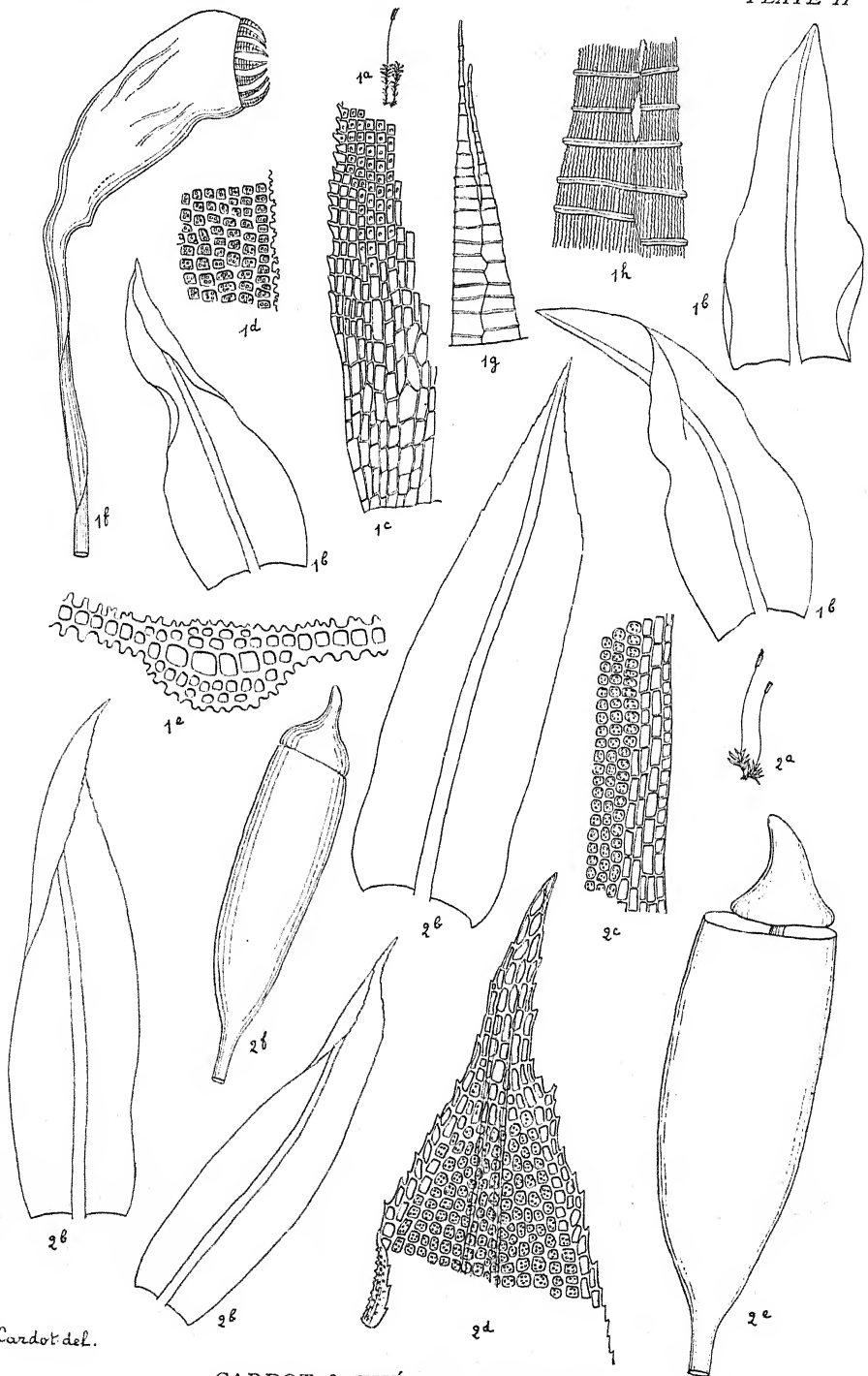
Nachet's objectives 3 and 6, oculars 1 and 3, with camera lucida. All drawings are reduced  $\frac{1}{4}$  in photo-engraving.)

PLATE II.—1. *Dichodontium Olympicum*. *a*, entire plant, nat. size; *b, b, b*, leaves  $\times 32$ ; *c*, basal areolation  $\times 135$ ; *d*, marginal areolation in the upper part  $\times 260$ ; *e*, transverse section of the nerve  $\times 260$ ; *f*, capsule ripe and deoperculate  $\times 26$ ; *g*, a tooth of the peristome  $\times 105$ ; *h*, part of the same in the upper part  $\times 285$ .—2. *Desmatodon systylioides*. *a*, entire plant, nat. size; *b, b, b*, leaves  $\times 32$ ; *c*, marginal areolation in the middle  $\times 135$ ; *d*, areolation of the upper part  $\times 135$ ; *e*, capsule ripe  $\times 32$ ; *f*, capsule unripe  $\times 32$ .

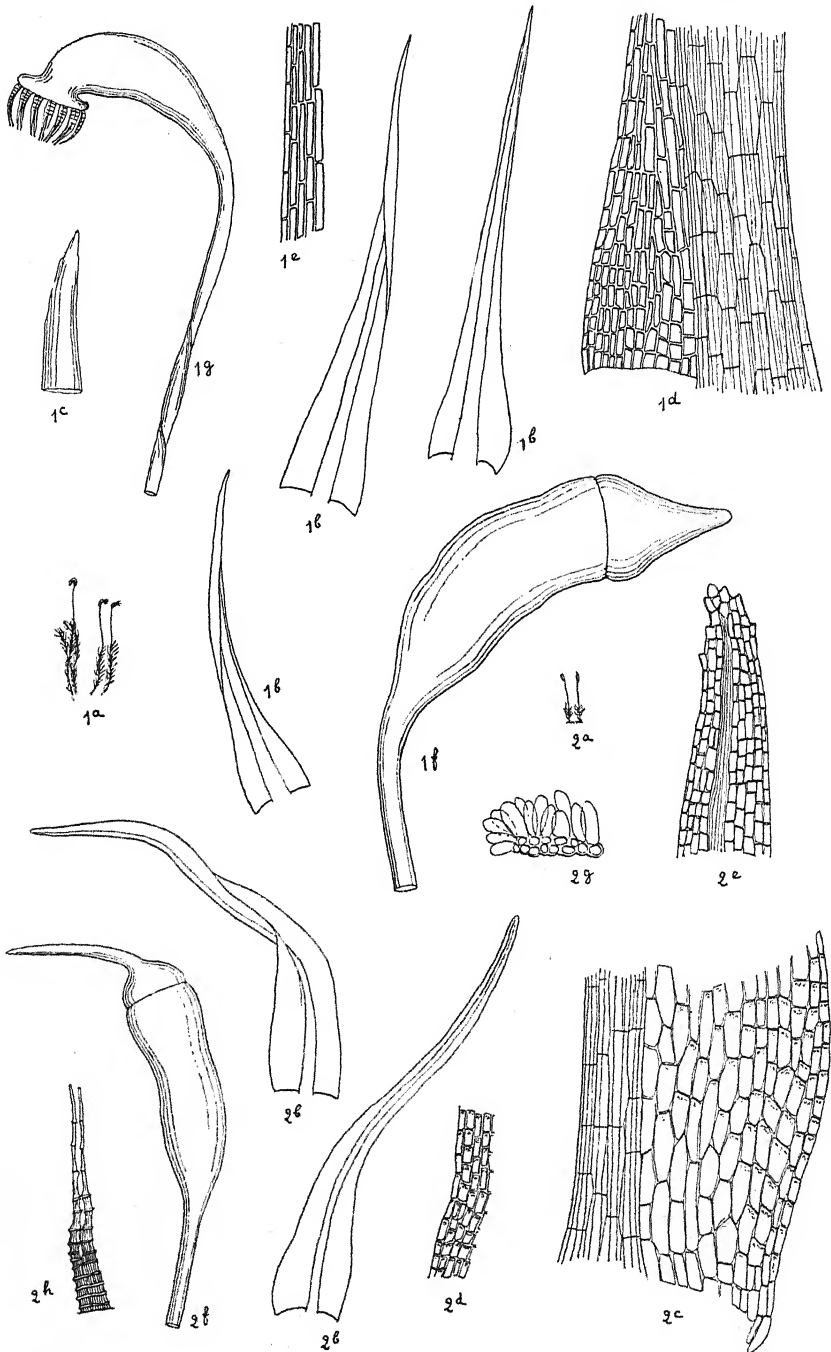
PLATE III.—1. *Dicranella Howei*. *a*, entire plant, nat. size; *b, b, b*, leaves  $\times 32$ ; *c*, apex of a leaf  $\times 135$ ; *d*, basal areolation  $\times 135$ ; *e*, marginal areolation in the middle of a leaf  $\times 135$ ; *f*, capsule in moist state  $\times 32$ ; *g*, capsule ripe and deoperculate, in dry state  $\times 32$ .—2. *Dicranella laxiretis*. *a*, entire plant, nat. size; *b, b*, leaves  $\times 32$ ; *c*, basal areolation  $\times 135$ ; *d*, marginal areolation in the middle  $\times 135$ ; *e*, areolation of the upper part  $\times 135$ ; *f*, capsule  $\times 32$ ; *g*, portion of the annulus  $\times 135$ ; *h*, a tooth of the peristome  $\times 135$ .

PLATE IV.—1. *Barbula eustegia*. *a*, entire plant, nat. size; *b*, the same  $\times 3$ ; *c, c, c, c*, leaves  $\times 32$ ; *d*, basal areolation  $\times 135$ ; *e*, areolation of the upper part  $\times 135$ ; *f*, external perichaetial leaf  $\times 32$ ; *g*, inner perichaetial leaf  $\times 32$ ; *h*, capsule  $\times 32$ ; *i*, peristome  $\times 60$ ; *j*, portion of a peristomial tooth  $\times 285$ .—2. *Grimmia pseudo-montana*. *a*, entire plant, nat. size; *b, b*, leaves  $\times 32$ ; *c*, basal areolation  $\times 135$ ; *d*, areolation of the upper part  $\times 135$ ; *e*, transverse section of the leaf in the lower part  $\times 135$ ; *f*, transverse section of a leaf in the upper part  $\times 135$ ; *g*, capsule ripe  $\times 26$ ; *h*, two teeth of the peristome  $\times 135$ .

PLATE V.—1. *Orthotrichum Idahense*. *a*, entire plant, nat. size; *b, b*, leaves  $\times 32$ ; *c*, basal areolation  $\times 135$ ; *d*, areolation in the upper part  $\times 135$ ; *e*, transverse section of a leaf  $\times 135$ ; *f*, capsule ripe and deoperculate, in dry state  $\times 26$ ; *g*, areolation of the capsular membrane in the upper part  $\times 135$ ; *h*, the same, in the lower part, showing a stoma  $\times 135$ ; *i*, two teeth of the peristome  $\times 135$ ; *j*, young calyptra  $\times 26$ .—2. *Bryum euryloia*. *a*, entire plant, nat. size; *b*, leaf  $\times 26$ ; *c*, marginal areolation in the middle of a leaf  $\times 135$ ; *d*, areolation of the upper part  $\times 135$ ; *e*, capsule in dry state  $\times 15$ .



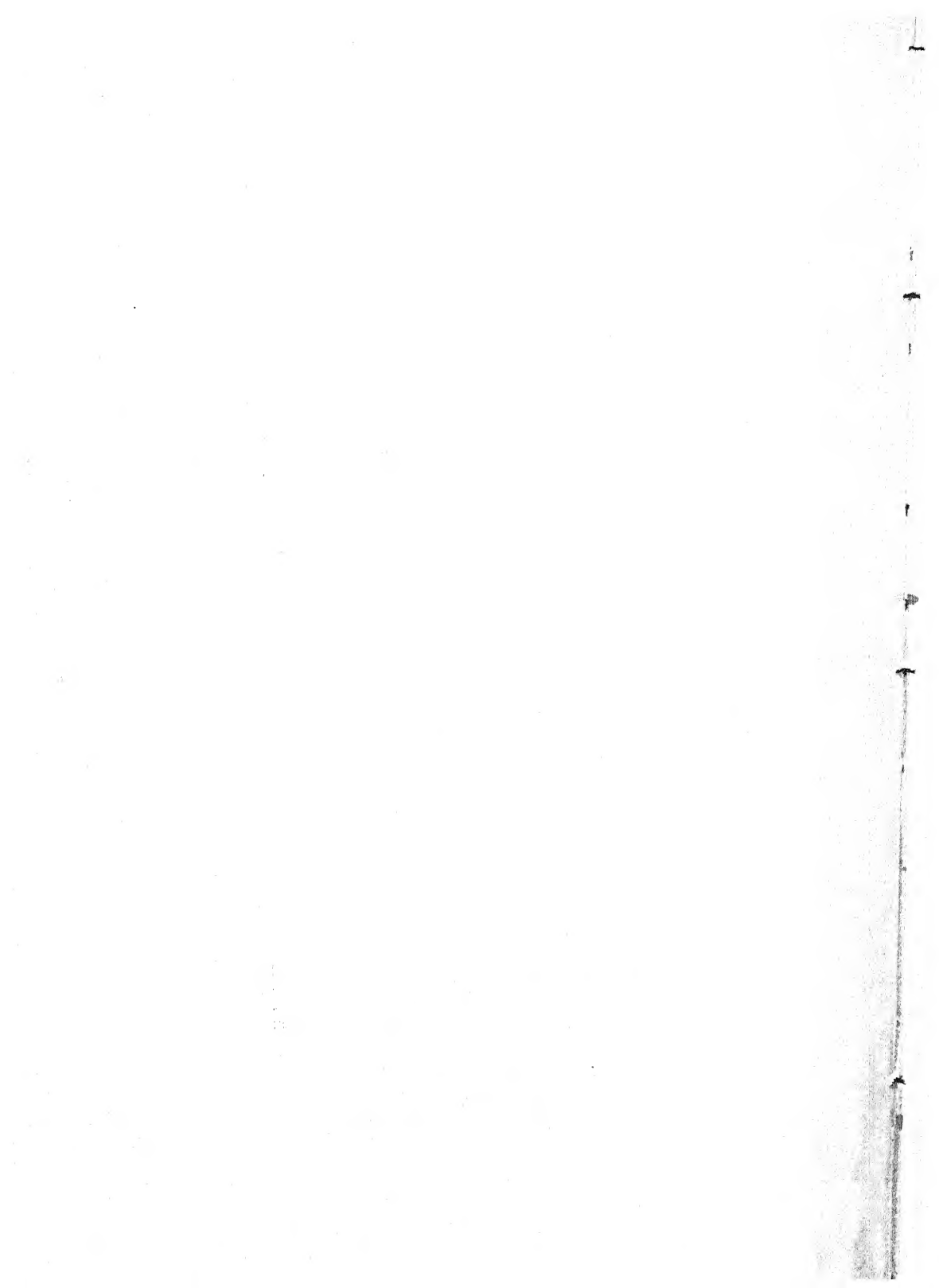


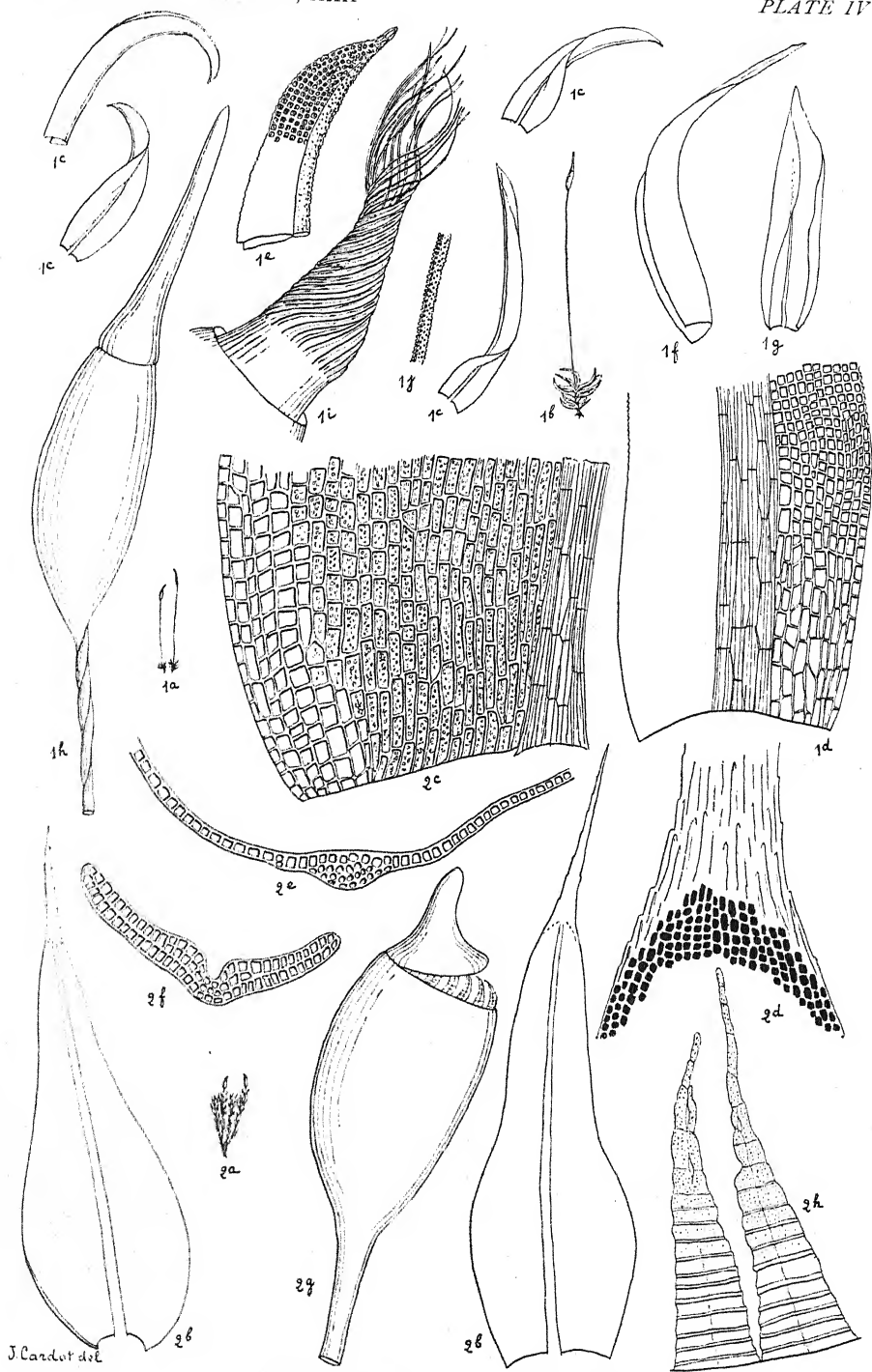


J. Cardot del

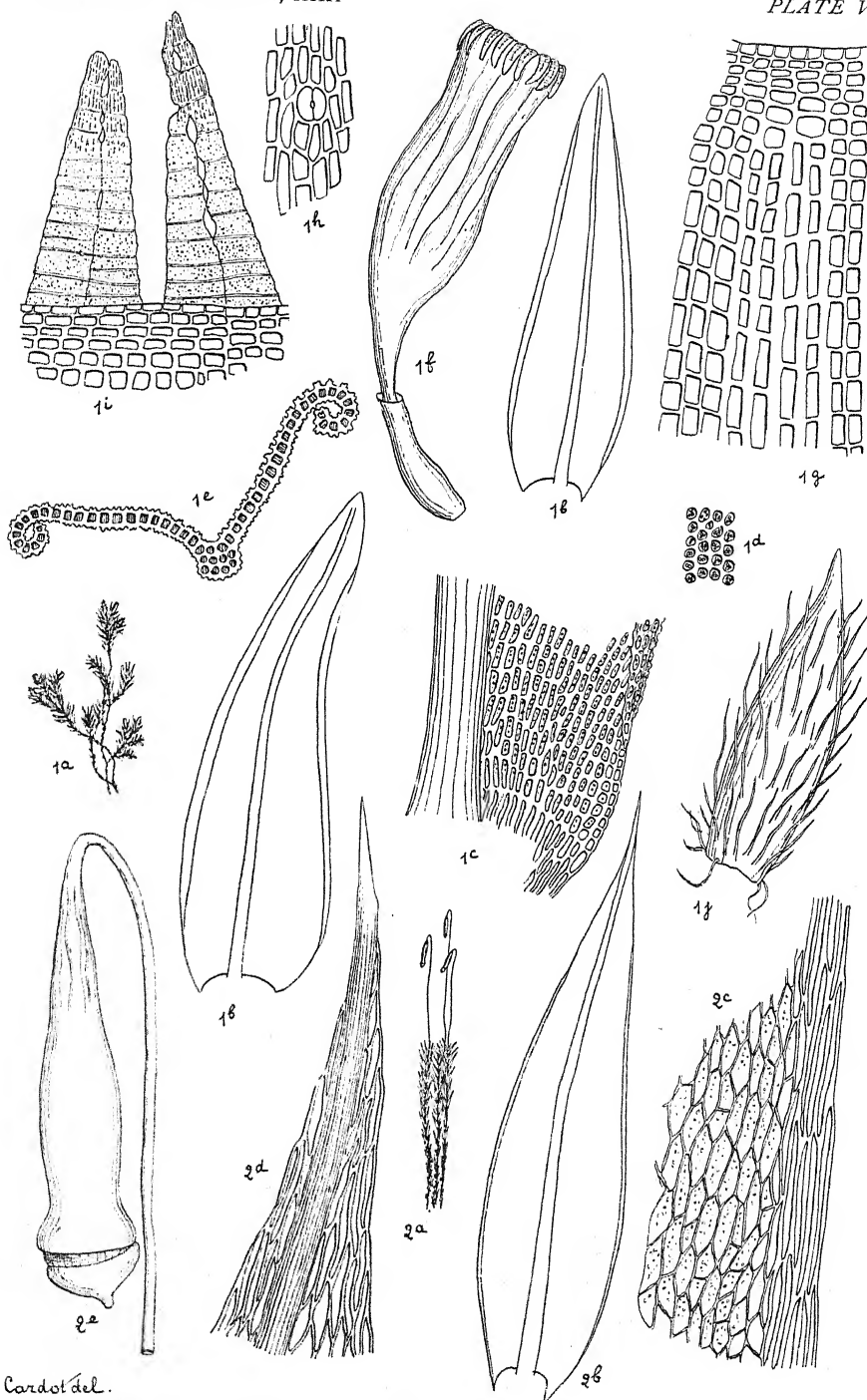
CARDOT & THÉRIOT on MOSSES











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# THE DEVELOPMENT OF THE EMBRYO-SAC IN SOME MONOCOTYLEDONOUS PLANTS.

KARL M. WIEGAND.

(WITH PLATES VI AND VII)

THE material for the following study was prepared in the ordinary way, by fixing in the chrom-aceto-osmic acid solution, imbedding in paraffin, and staining with the gentian-violet-orange combination. Although the development in *Canna* was found to be nearly normal, that in each of the other two plants showed some very interesting and important variations. Whether these throw any light on the problem of the homology of the embryo-sac can be determined only by more extended study of other plants.

## *Convallaria majalis* L.

### THE HYPODERMAL CELL AND ARCHESPORIUM.

The embryo-sac of *Convallaria* is derived from a hypodermal cell situated at the apex of the nucellus. This hypodermal cell is first discernible as an enlarged oblong or more or less distinctly triangular cell at the apex of the nucellus and directly underneath the epidermis; but can also be distinguished from the adjacent cells by its larger size and more granular contents. Very early in its development a single cell, the so-called "tape-tum," is cut off on the side adjacent to the epidermis. This immediately divides by an anticlinal wall into two daughter cells which lie side by side at the summit of the embryo-sac (*fig. 1*).

The nucellus is comparatively broad, and the growth of the archesporium taking place subsequent to the separation of the wall-cell is to a large extent in a lateral direction. To accommodate themselves to this the two daughter wall-cells undergo repeated anticlinal division, so that eight or ten cells are formed, all arranged in the same plane, and forming a plate of tissue just beneath the epidermis (*fig. 2*). The nuclei of

these cells increase somewhat in size until they are noticeably larger than those of the surrounding tissue, but unlike the sporogenous nuclei remain very dense. As the embryo-sac grows larger these cells are all pushed aside, with the exception of the more central ones which persist between the embryo-sac and the epidermis until after fertilization.

The other daughter cell resulting from the division of the primary hypodermal cell constitutes the archesporium. This immediately expands in all directions, partly at the expense of the ordinary tissue. At the time of the first nuclear division, the cell is oblong in shape and occupies a considerable portion of the nucellus. The nucleus during its period of growth passes through stages almost identical with those described for the microsporangial archesporium.<sup>2</sup> The chromatin network changes during synapsis (*fig. 2*) to a spirem ribbon (*fig. 3*), which later segments into the individual chromosomes. Corresponding stages in the nucleus of the pollen-mother cells and embryo-sac archesporium cannot be distinguished structurally; and this similarity is still farther emphasized by the synapsis occurring in each at the same stage in development.

#### THE FIRST NUCLEAR DIVISION.

Several good preparations of the first nuclear division were obtained, both in the nuclear plate and anaphase stages. In the plate stage the fibers are well marked; indeed the fascicles attached to the chromosomes are especially large and prominent. The spindle, like that of the pollen-mother cell, is rarely pointed at the poles, but is more often truncate (*fig. 4*).

The chromosomes are large oblong bodies arranged on the nuclear plate just as they are in the pollen-mother cell; that is, horizontally with one end directed away from the axis. Simultaneously the outer and inner ends commence to split longitudinally, but in perpendicular planes. In one case the separation is accomplished wholly without the aid of the spindle fibers; in

<sup>2</sup> WIEGAND: The development of the microsporangium and microspores in *Convallaria* and *Potamogeton*. Bot. Gaz. 28: 328. 1899.

the other the fibers seem to accomplish the separation. The former splitting is only partial, while the latter finally divides the chromosome into two V-shaped parts which move at once to the poles through the influence of the spindle fibers. This is therefore a heterotypic division, and similar to the one occurring in the pollen-mother cell. In fact it seems probable that having the spindle alone one could not distinguish the two cases, even after a careful study of the chromosomes themselves. The number of chromosomes was found to be eighteen, and since the number counted in the somatic cells was greater than thirty, it is evident that reduction takes place at this period. During the anaphase of the first division (*fig. 5*) a definite cell-wall is deposited, which divides the original cell into two nearly equal parts.

#### THE SECOND NUCLEAR DIVISION.

The nucleus in each of the two daughter cells resulting from the first division very quickly divides again, and it so happens that the two spindles are formed simultaneously. No cell-walls are formed after this division, at least not before the embryo-sac is nearly mature. At the stage shown in *fig. 8* four nuclei are present. The second division spindle was in this case directed longitudinally; therefore in the same plane as the first; but this is not always the case. Sometimes the axis of the spindle is inclined and in fact almost transverse. The position of the spindle, however, seems to be of little importance, since one may find the two daughter nuclei during later stages either one above the other or side by side.

The resting stage between the first and second divisions, like that in the pollen-mother cell, is very short. The V-shaped chromosomes remain distinct and uninclosed by a definite membrane. The spindle quickly forms, apparently from the surrounding cytoplasm, and at the same time the chromosomes are crowded toward the equator where they arrange themselves in a nuclear plate. These spindles were in all cases much less distinct than the first one, and appeared in the earlier stages



shorter and more truncate, while in the anaphase no cell-plate whatever was produced.

The way in which the chromosomes divide could not be positively determined. The segments on entering the nuclear plate, and even while in the plate as seen from the poles, are much curved (*fig. 6*). A side view of the nuclear plate shows a dense mass with projecting arms similar to the corresponding figure in the pollen (*fig. 6*). When the segments move to the poles they are seen to be quite long and straight, and never V-shaped as in the heterotypic division (*fig. 7*). After reaching the pole they at length fuse, and a membrane is formed around them, thus bringing about a truly resting condition again.

It seems scarcely to be doubted that the process here is identical with the second division in the pollen-mother cell. Indeed, every appearance in the one has its almost exact counterpart in the other.

#### GROWTH AND DEVELOPMENT OF THE EMBRYO-SAC.

The further history of the embryo-sac is very interesting, since it shows some deviations from the ordinary process in both monocotyledons and dicotyledons. The two superimposed daughter cells should probably be considered as constituting the so-called "axial row" in this case (*figs. 6-8*). At least, so far as the nuclei are concerned, they are the equivalents of the two cells first formed in *Canna*. There is therefore a two-celled axial row instead of a four-celled one, as in *Canna* and many other plants. It would be expected then that during development the lower cell alone would become the embryo-sac, while the upper would undergo dissolution as in nearly all other cases. This, however, is not the case.

At the time when the spindles of the second division occur, the archesporium as described above is two-celled. No walls are produced by the second spindles, as a result of which each cell now contains two nuclei. This stage is followed by a comparatively long period of growth in which both cells increase several times in size, as do also their nuclei. A large number of

preparations were obtained illustrating this condition and representing all stages up to the next division of the nuclei. As shown in the accompanying figure (*fig. 8*), the upper cell becomes gradually larger and more vacuolate, while the cytoplasm of the lower stains more deeply and fills nearly the entire cell cavity. No cases were observed, however, where one could infer that either cell was in the process of disintegration.

At a period shortly before the opening of the flower further changes occur, the first of which is the immense increase in size of the upper cell. The wall between the two still seems to remain intact, although becoming very thin and delicate. In some cases the wall is so delicate at this stage as to be almost invisible, and may have broken down entirely, in which case both cells would be merged into the general cavity of the embryo-sac, but in later stages two cells are again seen. All of the four nuclei undergo division simultaneously, resulting in the stage with eight nuclei, just as in *Lilium* (*fig. 9*). In several cases shortly after this two large nuclei were seen fusing near the lower end of the upper cell, while the lower cell contained the distorted remains of three other nuclei. The latter cell was thus apparently already in the process of disintegration. It seems probable that one of the four nuclei originally formed in the lower cell must have ruptured the thin cell-wall and fused with the upper polar nucleus after the normal manner.

In *fig. 10* is represented an embryo-sac just prior to fertilization. At this stage the "egg-apparatus" consists of two synergids and the egg. The former lie close together, with the long axis more or less transverse to the axis of the embryo-sac, and are elliptical in shape, with the wall at the upper end thickened and densely striate. Just below these is the large egg nucleus, separated from the synergids and from the main cavity by very delicate cell membranes which seem to extend completely across the embryo-sac so as to join the lateral walls on either side. Below the egg-apparatus is the very large main cavity of the embryo-sac lined with a thin layer of cytoplasm. In this, near the base, is the large definitive nucleus still showing signs of the

previous fusion of the two polar nuclei. At the base of the embryo-sac are the distorted remains of three antipodal nuclei still separated from the main cavity by a distinct cell-wall. It was impossible to determine whether this had been formed anew, or whether the old perforated wall had simply been repaired.

At the time when the main features of this study of *Convallaria* were read at the Boston meeting of the American Association for the Advancement of Science, so far as the writer was aware no exactly similar case had been observed. Mann<sup>2</sup> had discovered a cross wall in the embryo-sac of *Myosurus*, but here the development seems to have been somewhat different; and Strasburger<sup>3</sup> had figured a structure somewhat resembling a cross-wall in the embryo-sac of *Allium*, although it was mentioned in the text as a plasma-plate. Since that time, however, McKenney<sup>4</sup> in making a careful study of the embryo-sac of *Scilla* has described a process very similar to that in *Convallaria*. The archesporial nucleus underwent a period of growth before dividing. The first division showed the reduced number of chromosomes and was followed by a cell-wall. The two succeeding divisions in each daughter cell were not followed by a cell-wall, so that as a result two cells were present, each containing four nuclei. Up to this point the process was similar to *Convallaria*, but it was further found that only the upper cell took part in the formation of the embryo-sac, and not both as in *Convallaria*, while the lower cell disintegrated.

The process in *Convallaria* is strikingly similar to that in *Lilium*, and is probably to be considered as simply a modification of that method or a transition to it from the ordinary type with two or four cells in the axial row. It differs merely in the possession of a cross-wall, which however partially or

<sup>2</sup> MANN: The embryo-sac of *Myosurus minimus* L. Trans. and Proc. Bot. Soc. Edinburgh 29: 351. 1892.

<sup>3</sup> STRASBURGER: Die Angiospermen und die Gymnospermen (1879). Pl. 6. figs. 81-86.

<sup>4</sup> MCKENNEY: Observations on the development of some embryo-sacs. Contrib. Bot. Lab. Univ. Pennsylvania 2: [no. 1] 80. 1898.

entirely breaks down before the fusion of the polar nuclei, one of which comes from each cell.

**Potamogeton foliosus Raf.**

THE HYPODERMAL CELL AND ARCHESPORIUM.

In the earliest stage obtained the hypodermal cell at the apex of the nucellus had commenced its period of growth. No definite form can be ascribed to it at this period, although it is perhaps more often wedge-shaped. Even from the beginning it usually contains more protoplasm than the other cells, a feature which at a later stage becomes still more noticeable.

After a short period of growth this cell divides. The inner daughter cell resulting from the division becomes immediately the archesporial cell (*fig. 14*). The outer daughter cell divides again by an anticlinal wall. Two cells now lie side by side above the archesporium, as is represented in *fig. 12*, which shows a cross-section through the apex of the nucellus. *Fig. 13*, also a cross-section of the nucellus taken from the same inflorescence as *fig. 12*, shows that each of two cells now divides again, so that four daughter cells are formed all in the same plane. This last division may take place either before or after the first periclinal division of the wall-cell, commonly however before. Periclinal divisions in all four cells now begin, so that at length four rows of cells are formed between the archesporium and the epidermis. The process may continue until as many as six layers are produced; and these all persist until the embryo-sac reaches maturity, although often in a more or less compressed condition.

In the anther the hypodermal cell divides into two parts, one being destined to produce the archesporium, while the other after two or three periclinal divisions constitutes, together with the epidermis, the wall of the anther. The stages leading up to the production of the embryo-sac in the ovule are in many respects very similar to those occurring in the young anther. The hypodermal cell here also divides by a periclinal wall into two daughter cells, the innermost of which

becomes the archesporium, while the outer, the so-called 'tapetum,' forms a part of the sporangial wall.

In *Rosa*<sup>5</sup> and *Fagus*<sup>6</sup> the epidermis has been found to divide several times by periclinal walls, and thus to form a considerable portion of the tissue between the archesporium and the apex of the nucellus. The unusually large number of cells in this region in *Potamogeton* at first suggested that the same phenomenon might also be found here; but a careful study showed that no divisions of the epidermis beyond an occasional doubling of individual cells ever take place, and all of the tissue can be easily traced to the primary wall-cell. Moreover, the similarity to the process in the microsporangia is so evident as to require no other explanation.

#### THE FORMATION OF THE EMBRYO-SAC.

The lowermost cell formed by the first division of the hypodermal cell begins to enlarge at once, and must henceforth be considered as the archesporium. The whole process during the early stages of development is perfectly normal. The archesporial cell soon undergoes division resulting in an upper and a lower cell (*fig. 15*). These probably correspond to the two resulting from the heterotypic division in *Convallaria*, but the fate of the two cells we shall find is somewhat different either from that in *Convallaria* or in *Canna*. The first division is immediately followed by a second nuclear division in each of the daughter cells, but without the formation of a wall between the two nuclei (*fig. 16*). The uppermost cell now shows signs of disintegration, as indicated by the cytoplasm, which becomes more dense and also stains more deeply. The nuclei also lose their definite outline, and finally the whole cell becomes much compressed and flattened against the wall-cells above. In a very short time, indeed, it can be recognized only as a dark cap at the summit of the embryo-sac. Several preparations were

<sup>5</sup> STRASBURGER: *Die Angiospermen und die Gymnospermen* 14. Jena, 1879.

<sup>6</sup> BENSON: Contributions to the embryology of the Amentiferae. *Trans. Linn. Soc. II. Bot.* 3: 410. 1894.

obtained showing the various stages of this process with great clearness.

The lower cell on the other hand continues to enlarge. The two nuclei are usually located at opposite ends of the cell and are somewhat larger than those of the surrounding tissue (*fig. 17*). In the next stage observed both of these nuclei had undergone division, so that two were present at each extremity of the embryo-sac (*fig. 18*). At the antipodal end, from this stage onward, a small pouch begins to appear which contains the two lower nuclei and finally all of the antipodal cells. One of the two upper nuclei is now sometimes found slightly below the other, and nearer the center of the embryo-sac; but many other preparations show that this is merely temporary or abnormal, and that before the next stage is completed they are both located together at the apex of the cell cavity (*fig. 19*).

Very soon after the last nuclear division a cell membrane can be seen to form around the two nuclei at the micropylar end of the embryo-sac, thus enclosing them in a little pouch (*fig. 19*). The membrane constantly grows thicker, until at length it is a distinct wall, and the two nuclei are henceforth entirely separated from the cavity below. This seems to preclude entirely the possibility that a polar nucleus may pass down and fuse with one from the lower group of cells, although the latter remains in the general cavity of the embryo-sac until a much later period.

At a still later stage three nuclei instead of two are to be found in the micropylar enclosure (*fig. 20*). Two of these are small and differ but slightly from those of the surrounding tissue, while the third is much larger and located below close to the membrane. It seems probable that the two smaller nuclei are produced by the division of one of the two original nuclei, and are really to be considered as synergids; while the other and larger one is the egg derived directly from the other nucleus without division.

The two nuclei at the antipodal end of the embryo-sac after the pouchlike extension has commenced to form usually lie one

above the other and are both quite large, each containing a large chromatin mass in the center (*fig. 19*). The lower one now divides into three daughter nuclei, all very much smaller than the parent nucleus, and with the chromatin scattered instead of being aggregated in a ball. These are all to be considered as antipodal cells, and persist in the little pouch until after fertilization. The upper nucleus continues to enlarge somewhat until about the time of fertilization, when it undergoes division followed by a cell-wall, as a result of which one daughter nucleus is inclosed in a cavity at the antipodal end of the embryo-sac (*fig. 21*). This then must be considered as a fourth antipodal cell, since it is one of the four parts of the original lower nucleus. The other daughter nucleus is the polar nucleus, but becomes at once the mother cell of the endosperm, and immediately undergoes division until a parietal layer of endosperm is formed (*figs. 23, 24*). The large antipodal nucleus and those of the endosperm, like the original nucleus from which they sprung, are all large and contain very large central chromatin masses. This, in addition to their position at successive stages, points toward a common origin. The large antipodal nucleus continues to grow for some time and is at length very conspicuous. It is by far the largest in the ovule, and the very large deeply staining chromatin mass may be seen even after the embryo has reached a considerable size.

The nuclei in *Potamogeton* are all very peculiar, differing from the ordinary type in having the chromatin mostly aggregated in a ball at the center of the cavity, instead of being distributed on the linin network. Although several hundred slides were prepared, none contained spindles in the embryo-sac, and consequently the sequence of the divisions had to be determined by other means. The appearance of such a remarkable process of development, and one so different from those already described, although still strongly suggesting certain features of the process found by recent investigators in related plants, made it very important that every step should be verified as far as possible. For this reason the material was worked over several

times, until the steps became so clear as to leave no doubt in the mind of the writer that the description as given above is correct.

To summarize briefly: the mature embryo-sac consists of an egg and two evanescent synergids, each without a cell-wall of its own, but all contained within a pouchlike cavity separated from the general cavity by a delicate wall. One synergid usually disappears before fertilization. Next below is a large cavity containing the endosperm nucleus, and later the endosperm, derived without fusion from the lower polar nucleus. At the base are the four antipodal cells, three of which are very small and chromatic, and are descendants of the same nucleus, while one is very large and together with the polar nucleus is derived from another parent. The antipodals are separated from the main cavity by a membrane formed at the time of separation of the polar nucleus and the antipodal cell. The endosperm even after fertilization never becomes more than a parietal layer (*fig. 24*).

The investigation of plants of the same and nearly related orders has shown that the occurrence of the large antipodal nucleus is not a peculiarity of *Potamogeton* alone, but is characteristic of a whole group of Monocotyledons. Schaffner<sup>7</sup> found that in *Sagittaria* the development was normal up to the formation of the two synergids, egg, and three minute antipodal cells. He then found that the two polar nuclei fused in the ordinary way, after which the definitive nucleus underwent division. This division was always followed by a transverse wall enclosing one of the daughter nuclei in a chamber at the base; and this was the one which became so large at a later period. Sometimes several were found, thus showing that the large nucleus had divided at least once or twice. From the other daughter nucleus was produced the endosperm. The structure in question was therefore thought to be an endosperm nucleus, rather than an antipodal cell as in *Potamogeton*.

<sup>7</sup> SCHAFFNER: Contribution to the life history of *Sagittaria variabilis*. BOT. GAZ. 23: 252. 1897.



In *Naias* and *Zannichellia* Campbell<sup>8</sup> found an axial row of two or three cells the lower of which alone became the embryo-sac. The development was normal up to the point where the polar nuclei were formed. The free egg-nucleus and synergids and the minute antipodal cells were very characteristic; but no fusion of polar nuclei was observed, and he doubts if it ever occurs. The enlarged basal nucleus is always present from this stage onward, but is not separated by a wall from the main cavity as in *Potamogeton* and *Sagittaria*. Campbell believes with Schaffner that this is a product of the first division of the definitive nucleus, but also admits that it may be the lower polar nucleus alone, while the upper has gone to form the endosperm.

#### FERTILIZATION.

The stages representing the various steps in the process of fertilization are rarely met with in this plant. Several good preparations were obtained, however, and these will form the basis of the following description.

The pollen tube enters the embryo-sac through the micropyle at a point directly behind the egg. In its course it passes close to the partially disintegrated synergid, but the tube is quite slender and scarcely inflated after entering the embryo-sac, thus differing decidedly from *Sagittaria*, in which Schaffner described the inflation as very marked.

The two sperm nuclei were last noted in the mature pollen grain where they were both inclosed in the same cell-wall. They remain united even during their trip down the pollen tube to the embryo-sac and enter the egg together. Fusion of the egg and sperm nucleus was not observed. The stage immediately preceding this was found however, and is figured in *fig. 22*. Here the egg is easily recognized, and lying in close proximity to it, indeed even touching it, are the sperm nuclei. In reality only one actually touches the egg nucleus. The other lies at the side or even at the back of the first, and is already in the process of

<sup>8</sup> CAMPBELL: A morphological study of *Naias* and *Zannichellia*. Proc. Calif. Acad. Sci. II. 1:1. 1897.

disintegration. It seems therefore that in this case the two sperm cells never separate. At this stage the wall which separates the egg from the rest of the embryo-sac is prominent, and can easily be seen to inclose the synergids as well. Campbell found both sperm nuclei entering the embryo-sac in *Naias* but only one in *Zannichellia*. Schaffner found that in *Sagittaria* one always remained in the pollen tube.

#### THE EMBRYO.

The classical investigations of Hanstein<sup>9</sup> and Famitzin<sup>10</sup> on the development of the young embryos in both monocotyledons and dicotyledons have made us familiar with the process in a large number of plants. The work of later students has only gone to confirm the results reached by these investigators, at least as regards general features. However, the results obtained from certain monocotyledons by Schaffner and later by Campbell seem to be at variance with those above mentioned; and as bearing upon this point *Potamogeton* becomes of interest.

In *Potamogeton* the fertilized egg nucleus remains for a very short time in the resting condition, perhaps it divides immediately, but lack of spindles in the young embryos made it impossible to determine this. Immediately after the first division, the basal cell undergoes a change whereby it becomes gradually larger until a size several times the original is reached. At this stage the cell is a very striking object (*figs. 24, 26*). The very large vacuole, which soon appears, at length forces the nucleus to the bottom of the cell where it henceforth remains, while the nucleus itself undergoes considerable enlargement. Just behind this large nucleus of the basal cell, one can often see a synergid, even as late as the several-celled stage of the embryo.

The basal cell never undergoes division, but on the contrary remains for a long time in its enlarged condition attached to the end of the embryo-sac. But sometimes during the later stages

<sup>9</sup> HANSTEIN: Die Entwicklung des Keimes der Monokotylen und Dikotylen. Bonn, 1870.

<sup>10</sup> FAMITZIN: Embryologische Studien. Mém. de l'Acad. Imp. Sci. de St. Petersb. VIII. 26:—, 1879.

of development it may, together with the embryo, become entirely detached, so that the whole mass is then free (*fig. 26*). The wall increases slightly in thickness, and is always thicker than any other wall in the young embryo.

The first division of the egg is soon followed by a second and later by a third. The embryo then consists of a row of four cells of which the three upper have nearly the same size. Occasionally only three cells are present at this stage, but the four-celled stage predominates. The next division is vertical, and in the uppermost cell (*fig. 25*). This is again followed by one or two more oblique walls, thereby dividing the terminal cell into several sectors, each extending from the basal line to the periphery. Periclinal and anticlinal walls form successively in the different sectors, as a result of which the upper cell soon becomes a mass of tissue (*fig. 26*).

Meanwhile divisions have occurred in the next lower cell. This first formed an oblique wall, after which several divisions took place in various directions. The third cell from the apex has divided once or perhaps twice by vertical walls. The embryo now is nearly spherical, a fact which makes it very difficult to trace the development farther with the idea of determining just what portions of the mature embryo are derived from the various primary cells. The oldest stage at which one can with certainty distinguish the four original cells is shown in *fig. 26*.

The subbasal cell has here undergone a transverse as well as two vertical divisions. No further divisions took place in this cell, and owing to this fact there is little difficulty in recognizing the tissues arising from the subbasal cell even in the half-grown embryo. The eight cells remain undivided at the base of the embryo where they form a narrow neck or stalk; in other words they together with the basal cell form the true suspensor (*fig. 27*).

The fate of the other two cells is immediately lost. It can be inferred from their position that the cotyledon arises from the upper cell and the axis from the subapical. We cannot be

far wrong in making this interpretation. The half-grown embryo shown in *fig. 27* was the oldest stage obtained. At this time only the epidermis was differentiated, while the plerome is scarcely distinguishable. At the upper end of the figure may be seen the cotyledon; and at one side the plumule, arising in a depression at the apex of the hypocotyl.

Schaffner and Campbell both find that no division takes place in the enlarged basal cell after its formation. There is certainly none in *Potamogeton*. Probably the observation of Hanstein that such a division does take place in *Alisma* was inaccurate. That such a division does occur in some monocotyledons, however, is a well established fact as shown by Coulter<sup>22</sup> in his studies of *Lilium*. In this plant the basal cell undergoes both longitudinal and transverse divisions. The process in *Potamogeton* does not differ essentially from that in *Sagittaria* and *Naias*, as given by the two authors cited above. The subbasal cell in *Naias* divides by transverse walls into three instead of two cells, of which the upper forms several cells, while the lower remains undivided. In *Sagittaria* the subbasal cell divides also once more than in *Potamogeton*, and the uppermost daughter cells here again form considerable tissue.

### *Canna Indica* L.

#### THE HYPODERMAL CELL AND ARCHESPORIUM.

*Canna* represents still a third type of the monocotyledonous embryo-sac, differing in method of development from *Lilium* *Convallaria*, and also from *Potamogeton*.

The hypodermal cell very soon divides into two parts by means of a periclinal wall (*fig. 29*). The upper cell then by repeated anticlinal division rapidly forms a layer of about nine cells directly above the future embryo-sac. This layer together with the epidermis constitutes the wall of the sporangium, and remains unchanged until finally displaced by the embryo-sac beneath.

<sup>22</sup> COULTER, J. M.: Contributions to the life-history of *Lilium Philadelphicum* — The embryo-sac and associated structures. BOT. GAZ. 23:413. 1897.

Meanwhile the lower of the two cells formed by the first division of the hypodermal cell has undergone considerable growth. It rapidly becomes several times its original size, and extends nearly to the base of the nucellus; but the increase in breadth is not so great. Just previous to the first division of its nucleus the cell is unusually long and narrow, but is completely filled with cytoplasm (*fig. 29*).

The cell gradually becomes longer and longer until at length a division takes place whereby two daughter cells are formed (*fig. 31*). After a short time both of these daughter cells divide again simultaneously. As a result we have an axial row of four cells reaching from the base to the apex of the nucellus (*fig. 32*). In the upper three cells a change is noticed almost at once, whereby the cytoplasm becomes denser and darker, the nuclei less chromatic, and the cells in fact show evident signs of disintegration (*fig. 33*). The lower cell by its continued growth gradually compresses the other three, which very soon are all crowded into a small disorganized mass in the micropylar region (*fig. 34*). A large number of sections was obtained showing all stages of this process in the plainest manner. The lowermost cell alone finally takes part in the formation of the embryo-sac. The process in *Canna*, therefore, is exactly in accord with that in the Iridaceæ, Rosaceæ, Polygonaceæ, and many Ranunculaceæ and Liliaceæ as described by Strasburger and others.

Owing to the extremely narrow cavity and small nuclei, the changes within the embryo-sac are very difficult to follow. Humphrey<sup>22</sup> has already given a full discussion of the literature on the embryo-sac of *Canna*; and also the results of his own investigations on the same plant. In this case the antipodal cells were not discovered, and he came to the conclusion, as did also Guignard<sup>23</sup> that these cells although always formed must disintegrate immediately.

<sup>22</sup> HUMPHREY: The development of the seed in the Scitamineæ. *Annals of Bot.* 10:1. 1896.

<sup>23</sup> GUIGNARD: Recherches sur le sac embryonnaire des Phanerogames Angiospermes. *Ann. Sci. Nat. Bot.* VI. 13:136. 1882.

From the material at hand it appears that a short time after the last division in the axial row, the primary nucleus of the embryo-sac, *i. e.*, of the lower cell, divides, and one of the daughter nuclei passes to each end of the already much elongated cavity. The spindles representing the latter division were not obtained, but several cases were found where there were two nuclei at each end of the embryo-sac, and still others where there were four (*fig. 34*). One or two sections showed the three antipodals, two synergids, and the egg; while near the center of the cavity was a very large nucleus, apparently the definitive nucleus of the embryo-sac. The fusion of the polar nuclei was therefore not observed, but probably took place as indicated by the two nucleoli and cross line in *fig. 35*. Just previous to fertilization the egg-apparatus was found to be separated from the main cavity by a delicate membranous wall, and to consist of two very small partially disintegrated synergids located very near the micropyle, and a much larger egg nucleus suspended some distance below, as in *fig. 35*. At this stage the antipodals are often in an advanced stage of disintegration, and are more or less clearly separated by a delicate membrane from the cavity above.

*Canna* differs from *Convallaria*, therefore, principally in the embryo-sac being formed from one cell of the axial row, and one element of the division into four of the mother nucleus. In the latter plant the whole axial row and all four elements of the division of the mother cell go to form the embryo-sac.

#### THE NUCLEAR DEVELOPMENT.

At a very early stage the nucleus of the archesporial cell passes into the condition of synapsis, in which as usual the linin is massed together at one side of the nuclear cavity. After a time indications are seen of the gradual loosening of the knot, with a simultaneous migration of the spiral coils to the more distant parts of the nucleus. The nucleolus here, as in *Convallaria*, is seen to remain intact during the whole process. It stains much deeper with the gentian-violet than does the chromatin,

and can therefore be readily distinguished. The spirem itself is composed of comparatively few turns of the rather broad chromatin thread, and is made up of alternate segments of chromatin and linin (*fig. 29*). The nucleus lies imbedded in the cytoplasm, which at this stage completely fills the cell cavity. It is now very large, and several times the size of those of the adjacent vegetative cells, and from it numerous radiations extend into the surrounding cytoplasm.

The nuclei and chromosomes in *Canna* are so small that little could be done toward working out the segmentation of the latter. Some features of the nuclear division, however, may be noted.

All of the nuclei in this plant possess a true nucleolus, as in *Convallaria*, and a very meager linin net-work on which the small amount of chromatin is unequally distributed. In the vegetative nuclei at the time of division the chromatin becomes aggregated into six spherical masses lying just beneath the membrane, and the nucleolus at the same time disappears. The spindle now forms, and the ordinary process of division assures six daughter chromosomes for each resulting nucleus. This count was made many times with great ease, owing to the small number of segments, and always with the same result.

When the archesporial nucleus has reached its limit of growth the chromatin of the spirem ribbon seems to become aggregated into a number of globular masses, as in the vegetative nuclei, except that the number in many cases seems to be more than six. Only two spindles were found representing the heterotypic division, and these both showed the globular daughter segments on their way to the poles (*fig. 30*). The four counts here made gave in every case the number as six, instead of three as one would expect after reduction. The spindles of the second division both occur at the same time, and one or two sections containing these were also obtained (*fig. 31*). They were all in the nuclear plate stage, and here the number was actually three; but each chromosome seemed to be composed of two parts, one of which was located directly above the other

and more or less completely joined to it. The most plausible explanation for this seems to be that the segmentation for both divisions was nearly or quite completed before the formation of the first spindle, and that when they appeared on the nuclear-plate of the second spindle the segments had come together in pairs only to be separated again on going to the poles. No true resting stage seems to intervene between the two divisions. The above results are especially interesting, since three for the reduced number of chromosomes is one of the smallest so far found in plants. The later divisions in the embryo-sac were not observed. The spindle seems to be formed simply by the elongation of the kinoplasmic mass, no multipolar condition being noticed, and the mature spindle is long and slender but usually with obtuse poles. After each division a distinct membrane is deposited at the cell-plate, thus forming the axial row of four cells. The resting nuclei often show very distinct radiations from the nuclear membrane; especially is this the case with the lower one which now commences another period of growth before division in the embryo-sac.

#### SUMMARY.

*Convallaria*.—The hypodermal cell dividēs into an upper and a lower cell, of which the inner cell becomes the archesporium and the upper forms part of the wall. This is also the case in *Potamogeton* and *Canna*.

The stages of the growth and development of the archesporial nucleus are identical with those of the nuclei of the micro-sporangial archesporium.

The first division of the archesporial nucleus is the heterotypic division and corresponds to the first pollen-mother cell division in every respect.

The two spindles accompanying the second division are formed simultaneously. In appearance this division is identical with the second pollen-mother cell division and quite different from either the vegetative or heterotypic. A transverse division of the chromosomes could not be demonstrated.



Only the heterotypic division is followed by a cell-wall, and thus an axial row of two cells is formed each containing two nuclei.

Shortly after anthesis the transverse wall disappears and the four nuclei all divide simultaneously, producing four daughter nuclei at each end of the embryo-sac. The endosperm nucleus is formed by the fusion of one nucleus from each group. After the transverse wall is destroyed, therefore, the process is the same as in *Lilium*.

The number of chromosomes in the vegetative nucleus is about thirty-six. During the heterotypic and so-called "reducing" divisions eighteen may be counted. The apparent reduction therefore takes place prior to the first division of the archesporium.

*Potamogeton*.—The first division of the archesporial nucleus is followed by a cell-wall, but the second is not; so that an axial row of two cells, each containing two nuclei, is produced as in *Convallaria*.

The lower cell forms the embryo-sac, while the upper disintegrates.

Four nuclei are formed in the lower cell; the two at the upper end are at once enclosed by a cell membrane, and from them develop the two evanescent synergids and the egg.

The lower remain free. From one of these all three of the small chromatic antipodals are probably formed. The other divides, forming the fourth antipodal cell and the polar nucleus with a cross-wall between the two. The polar nucleus becomes the endosperm nucleus without fusion.

The mature embryo-sac contains two small synergids and a large egg nucleus enclosed by a wall near the micropyle; a very thin parietal layer of endosperm; and four antipodal cells enclosed by a transverse wall at the lower end of the embryo-sac, of which three are very small and one is very large.

The nuclei of *Potamogeton* are peculiar in having most of the chromatin aggregated in a ball at the center of the cavity.

At the time of fertilization the two sperm nuclei lie together near the egg nucleus, but only one is really in contact with the latter.

The fertilized egg-cell undergoes at first three divisions, forming a row of four cells. The terminal cell and the one next below give rise to the greater part of the embryo. In the subbasal cell only one transverse and two vertical divisions ever occur, and the cells thus formed together with the very much enlarged basal cell form the suspensor.

*Canna*.—The heterotypic division is also the first division of the archesporial nucleus, and is followed by a transverse wall.

The second divisions occur simultaneously and are also followed by cell walls. These form an axial row of four cells.

The lower cell alone gives rise to the embryo-sac; the other three finally disintegrate and disappear. The further development is quite normal.

The nuclei of *Canna* are more nearly like those of *Convallaria* than *Potamogeton*. They have a true nucleolus and no central chromatin mass.

The number of chromosomes in the vegetative divisions is six. When passing to the poles at the heterotypic division there were still six; but later the second division showed only three as the reduced number. Probably the segmentations for both divisions occur during the prophase of the heterotypic division. This number is one of the smallest yet found in vegetable tissue.

CORNELL UNIVERSITY.

#### EXPLANATION OF PLATES VI AND VII.

Figures 1-11. *Convallaria majalis* L.

FIG. 1. A vertical section through a young ovule, showing the epidermis, archesporial cell, and two of the wall-cells between; the archesporial nucleus is in the resting stage.

FIG. 2. The same at a later stage; the wall-cells have undergone further division, and the archesporial nucleus is in synapsis.

FIG. 3. The archesporial nucleus in the spirem stage; the ribbon contains small denser portions of chromatin.

FIG. 4. Nuclear-plate stage of the first or heterotypic division of the archesporial nucleus; the chromosomes appear + -shaped, as in the pollen mother-cell.

FIG. 5. Anaphase of the same division; the V-shaped segments have reached the poles, and the cell-plate is forming.

FIG. 6. The nuclear plate stage of the second or so-called "reducing" division; the chromosomes are arranged very differently; between the two spindles is the wall formed during the previous division.

FIG. 7. Anaphase of the same division.

FIG. 8. A later stage showing the four nuclei thus formed.

FIG. 9. Each of these nuclei now divides forming four at each end of the embryo-sac; the cross-wall has disappeared.

FIG. 10. The mature embryo-sac ready for fertilization; over the apex is the epidermis and the remains of the other wall-cells; the egg-apparatus consists of the large egg nucleus and two striated synergids; at the base are the disorganized antipodal cells enclosed by a cell wall, and above is the endosperm nucleus.

FIG. 11. A spindle from the vegetative tissue, nuclear-plate stage; the chromosomes extend in all directions.

Figures 12-27. *Potamogeton foliosus* Raf.

FIG. 12. A cross section of the apex of the nucellus, showing at the center the two wall-cells lying above the archesporium.

FIG. 13. Same in the four-celled stage.

FIG. 14. Vertical section of the nucellus, showing the archesporial cell with resting nucleus, and one wall-cell above.

FIG. 15. The axial row of two cells; the cross-wall was formed after the first or heterotypic division of the archesporium.

FIG. 16. Same, later stage; the "reducing" division is not followed by a cell-wall.

FIG. 17. The lower cell developing into the embryo-sac; the upper cell forms a crushed mass above.

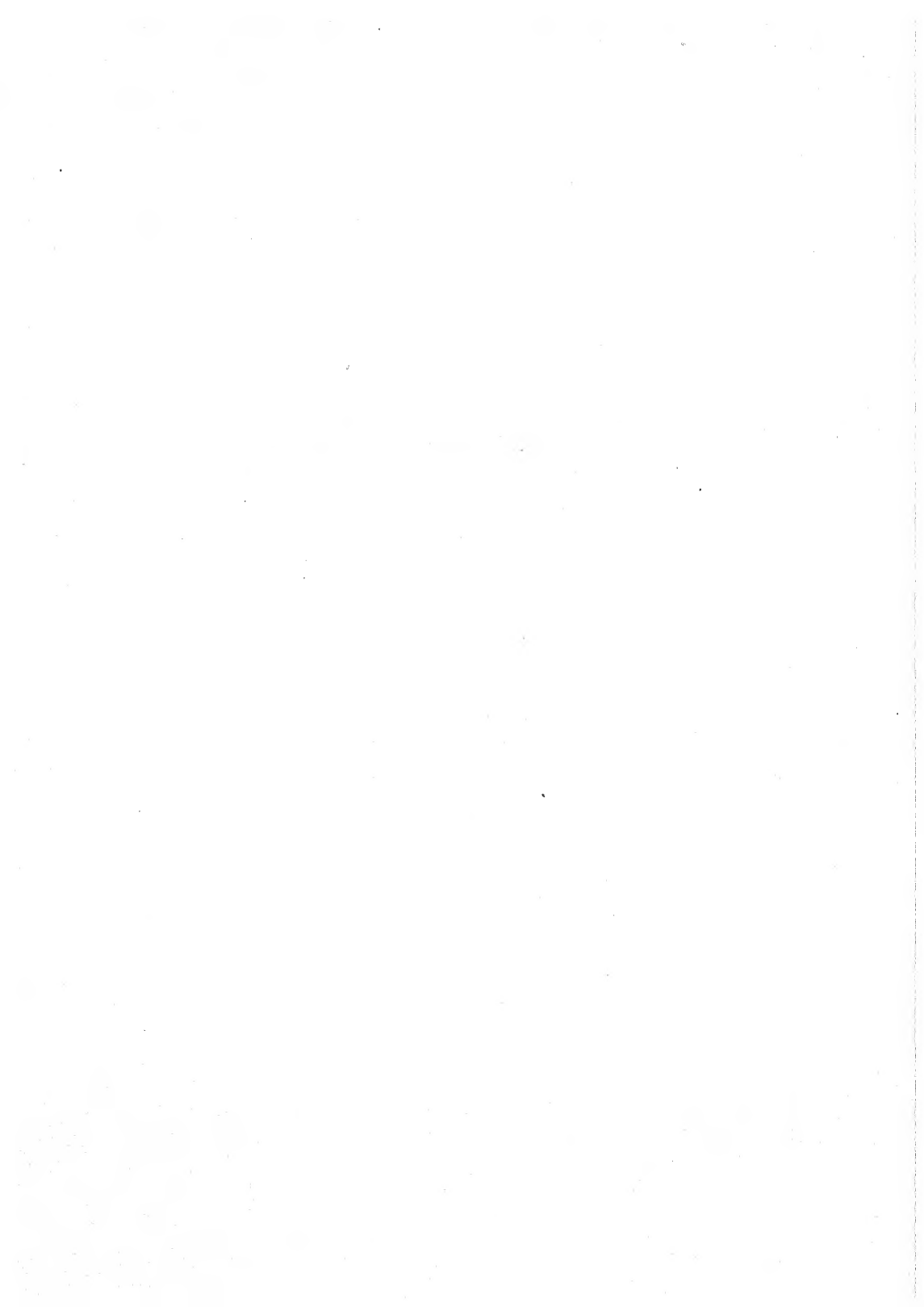
FIG. 18. Same, but each nucleus has divided again.

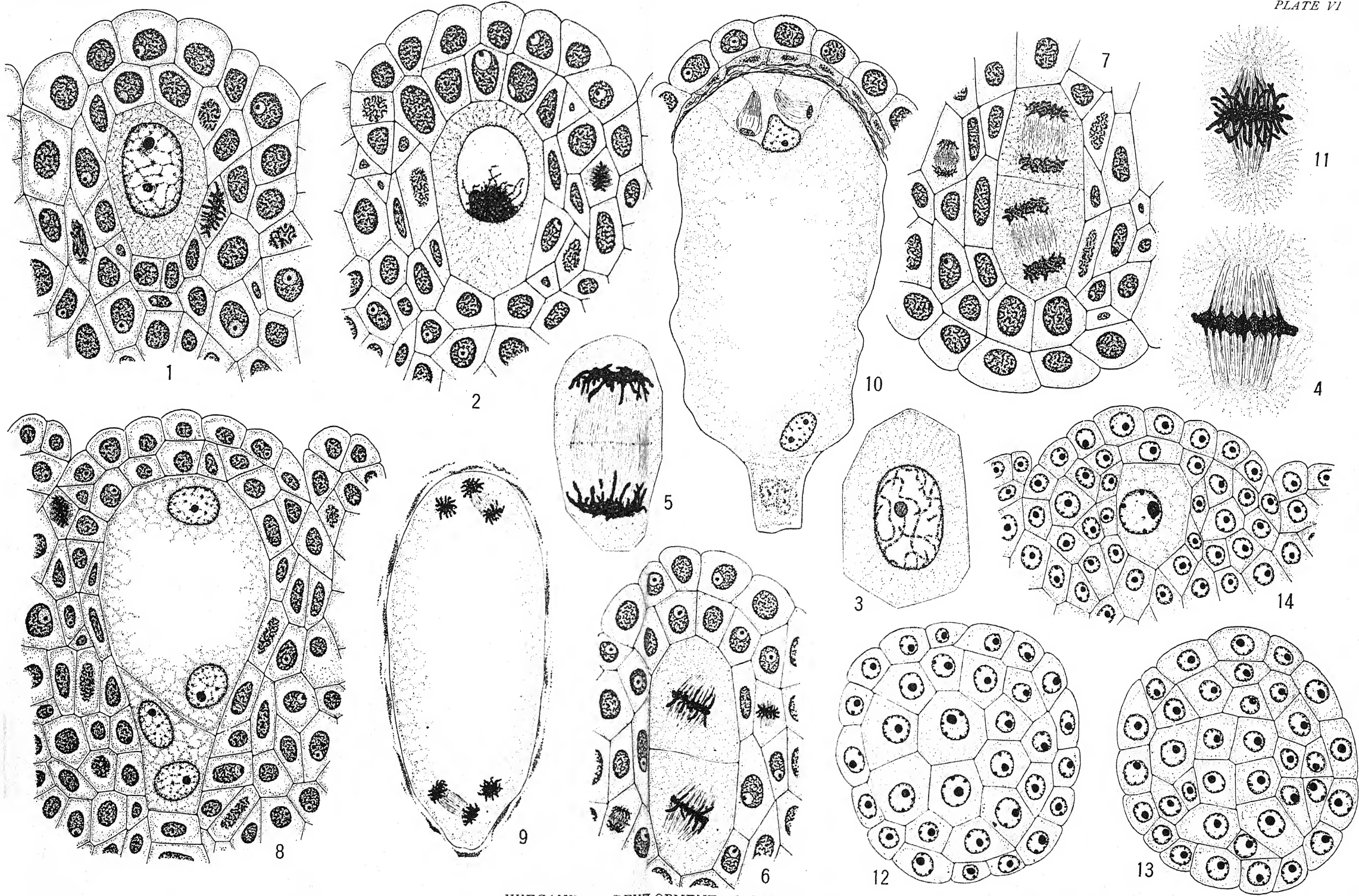
FIG. 19. Same, with a wall forming just below the two upper nuclei, separating them from the cavity below.

FIG. 20. The young embryo-sac with the large egg and two small synergids enclosed by a cell-wall, three small antipodal cells at the base and a very large nucleus above.

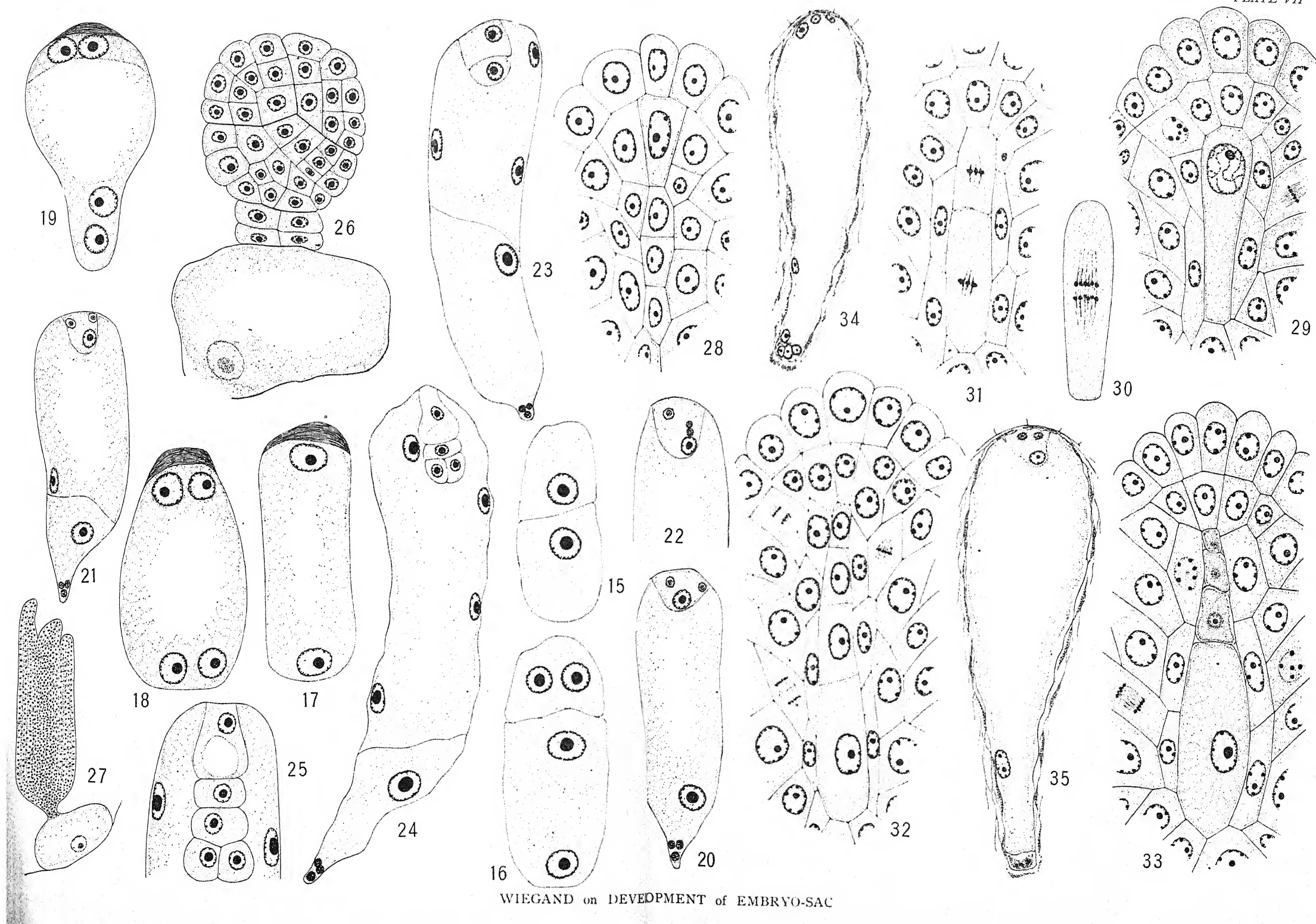
FIG. 21. Same, slightly older; the large nucleus has divided into a polar nucleus above and a large antipodal nucleus below separated by a cell-wall: the former becomes immediately the definitive nucleus.

FIG. 22. Egg apparatus showing a stage in fertilization; the two sperm nuclei lie near the egg, and one synergid above is in the process of disintegration.





WIEGAND on DEVELOPMENT of EMBRYO-SAC



WIEGAND on DEVELOPMENT of EMBRYO-SAC





FIG. 23. The embryo in the two-celled stage; below are three endosperm nuclei and the antipodal cells; this is an abnormal case in which the cross-wall was formed up near the center of the embryo-sac.

FIG. 24. A complete embryo-sac containing a young embryo in which the first oblique wall is in the third instead of the fourth cell.

FIG. 25. A young embryo showing the first oblique wall in the fourth cell as is normally the case.

FIG. 26. The embryo at a later stage; the basal cell has become much enlarged; the subbasal cell forms the remainder of the suspensor, four cells only are here shown; the heavy line through the embryo separates the apical from the subapical cell, and in the former the oblique wall can also be distinguished; the other divisions are irregular.

FIG. 27. The oldest embryo obtained; the basal cell and the derivatives of the subbasal cell still persist; the cotyledon and plumule are differentiated as is also the central cylinder.

Figures 28-35. *Canna Indica* L.

FIG. 28. A vertical section through the nucellus showing the hypodermal cell slightly larger than those of the surrounding tissue.

FIG. 29. Same at a later stage; the hypodermal cell has given rise to the archesporial cell and to a primary wall-cell which in turn has divided into three or four daughter cells; the archesporial cell is already much enlarged and the nucleus is in the spirem stage.

FIG. 30. The first or heterotypic division of the same nucleus in metaphase; six segments in each group may be counted.

FIG. 31. The second division of the archesporial nucleus in the nuclear-plate stage; three chromosomes only are here present; the cross-wall was formed during the previous division.

FIG. 32. The axial row of four cells; the central cross-wall was formed at the heterotypic division, the upper and lower at the second division.

FIG. 33. The axial row with the three upper cells in the process of disintegration; the lower is growing to become the embryo-sac.

FIG. 34. The embryo-sac showing two synergids and the egg nucleus at the upper end, and three antipodals with the polar nucleus at the base; the upper polar nucleus has traveled part way down the lateral wall.

FIG. 35. Same at a later stage; the egg-apparatus is inclosed by a delicate membrane as are also the disintegrating antipodals; the two polar nuclei have fused to form the definitive nucleus.



## BRIEFER ARTICLES.

### SOME OBSERVATIONS ON APPLE TREE ANTHRACNOSE.

(WITH TWELVE FIGURES)

FOR several years past the apple orchards of the Pacific northwest, including western Oregon, Washington, and British Columbia, have suffered seriously from the attacks of a fungous disease which has been known locally as "canker," "dead spot," or "black spot."

Although of considerable economic importance, the disease seems to have been entirely overlooked by mycologists, and nothing of importance concerning its nature has been recorded. When it was announced by Mr. Paddock, of the New York Experiment Station at Geneva, that *Sphaeropsis malorum* the cause of the well-known "black rot" of the apple and quince, is also the cause of the bark disease of apple trees, it was hoped that his discoveries would explain the cause of the similar western disease, but only a cursory examination was needed to show that this is not the case. Recently, with Mr. Paddock, I have had the privilege of comparing the two diseases with the result that we were both convinced that they are entirely distinct.

In deciding to ignore the term "canker," the most commonly used of the local names which have been applied to the disease, and in proposing for it the name of *apple tree anthracnose*, I hope to avoid confusion in the designation of the disease in the future. The term canker is most commonly used in European works on plant diseases to designate injuries to the bark caused by the various species of *Nectria*; and in the eastern United States it has been applied by Mr. Paddock to a somewhat similar disease of apple bark caused by *Sphaeropsis malorum* Peck. The term anthracnose, while it has perhaps no definite botanical significance, seems appropriate from the fact that the fungus which causes it, and for which we here propose the name *Gloeosporium mali-corticis* is closely related to numerous other fungi of economic importance which have quite generally been designated as anthracnoses.

Apple tree anthracnose attacks principally the smaller branches—those under two or three inches in diameter—although it also occurs

upon the larger ones. It appears first in the fall, soon after the autumn rains begin, as small, irregular, brown, sometimes slightly depressed, areas of the bark. During the fall and winter months it spreads but slowly, but with the advent of warmer weather in spring, growth takes place rapidly until the disease has invaded an area sev-



FIG. 1.

FIG. 2.

eral inches in diameter. Such areas under observation at Corvallis, Oregon, the past season cease to enlarge late in May, and early in June the first evidence of spore formation was seen. At that time the diseased areas were dark brown in color, markedly depressed, and in most instances limited by ragged, irregular fissures which separated the dead from the surrounding living tissues (*figs. 1, 2*). These dead spots vary in size from those not more than one half inch in diameter to extensive areas two or three inches wide by six or eight inches long. Occasionally a single area completely girdles a branch, thus killing at once its distal portion; but more commonly only a dead spot occurs,



FIG. 3.

from which in the course of a few months the bark sloughs off, leaving an ugly wound which requires several years to heal. When these wounds are at all numerous the branches are exceedingly rough and disfigured, and are also greatly weakened.

Early in June the first acervuli were observed. They appeared as small conical elevations of the epidermis, and were scattered irregularly over the diseased area. By the end of June these elevations had increased considerably in size, and in a few instances the overlying epidermis had been ruptured so as to expose the cream colored conidial mass. Material collected at that time and taken by me to Cornell University, where it was examined about the middle of July, revealed the presence of a few conidia, none of which, however, could be induced to germinate. In material which was collected in July, but which was not examined until early in October, the conidia were more abundant, but in dilution cultures in potato-agar only two spores were observed to germinate. However, material which was collected at Corvallis, October 4, and which reached me a week later, had developed numerous conidia which germinated readily both in water and in nutrient agar cultures. It would appear, therefore, that although evidences of the formation of acervuli may be noted early in June, mature conidia are not present in quantity before August or September.

Sections through a mature acervulus (*fig. 8*) show a subepidermal stroma from which arise comparatively long closely compacted basidia, on which the elliptical curved conidia are borne. As growth proceeds the overlying epidermis is ruptured and the

mature conidia are set free. A true pycnidium is not developed. When first exposed the conidial mass is a delicate creamy tint, but with age its outer surface becomes dark colored or even black. The conidia (*fig. 9*) are continuous, hyaline or with a greenish tinge, elliptical, curved, coarsely granular, and measure  $5-7 \times 16-28\mu$ . They average about  $6 \times 24\mu$ .

Late in July dilution cultures were made in neutral and in acid potato-agar from material that had been collected the last of June. In these cultures not a single colony developed. October 4 similar cultures were made from material which was collected about the middle of July. In this but two conidia could be found that had germinated. In fact so few conidia had developed that it was difficult to obtain enough for satisfactory cultures without obtaining an extensive variety of contaminating growths. To obviate this difficulty, on October 6 conidia from a single acervulus were carefully removed with a flamed scalpel, teased out in a drop of sterilized water, and transferred with a sterilized camel-hair brush to marked places on plates of acid potato-agar.

These plates were examined daily, and although numerous spores were seen none were observed to germinate until October 10, when two which had made a feeble growth were transferred to tubes of sterilized bean stems. At the time the general failure of the spores to germinate was thought to be due to their loss of vitality through having been kept too long in the laboratory; but it now appears to have been because the conidia used were not mature, since spores from material collected October 4 have continued to germinate readily up to the middle of November.

In cell and in Petri dish cultures in potato-agar the conidia germinate readily in about twelve hours at a temperature of  $22^{\circ}\text{C}$ . At  $29^{\circ}$  the germination is retarded indefinitely, although spores in cell-cultures

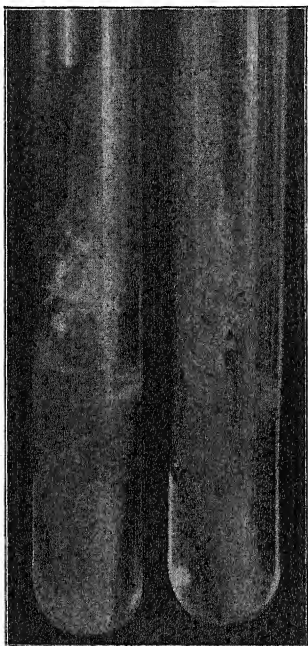


FIG. 4.

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which had been kept at this temperature in the thermostat for 24 and 48 hours germinated as usual when removed to the lower temperature. A germ-tube is developed at one end of the conidium (*fig. 10*) and the protoplasm begins to flow into it. Soon another tube pushes out, usually from the opposite end of the spore, and this is followed by others until from two to five tubes have been produced. In nearly all



FIG. 5.

FIG. 6.

FIG. 7.

instances there is a slight bulbous enlargement at the origin of each tube, much as is *Gloesporium nervisequum*, but less marked.<sup>1</sup>

Growth is comparatively slow, and at 24 hours from the time of sowing the germ-tubes are rarely more than twice the length of the spore. Even at this early stage, however, the production of secondary conidia has begun (*figs. 11, 12*). These are generally produced acrogenously from short lateral outgrowths of the germ-tubes, or of the conidium much as described for *Gloesporium fructigenum*,<sup>2</sup> and for *Colletotrichum gossypii*.<sup>3</sup> The branches are, however, much shorter, and

<sup>1</sup> STONEMAN, BERTHA: A comparative study of the development of some anthrac-noses. BOT. GAZ. 26:69. *pl. 12*. 1898.

<sup>2</sup> *Ibid.*

<sup>3</sup> ATKINSON, G. F.: Anthracnose of cotton. Jour. Myc. 6: 173-178. 1891.

numerous instances have been observed in which no such outgrowths could be seen, the secondary conidia having every appearance of being given off directly from the germ tube or even from the conidium itself. They are at first hyaline, later with a greenish tinge, granular, elliptical, rarely slightly curved, and always, so far as observed, decidedly smaller than the original conidium, although varying greatly with the character of the food supply. The most abundant production of these secondary conidia is in the immediate vicinity of the conidium, so that there is a tendency to produce an acervulus.

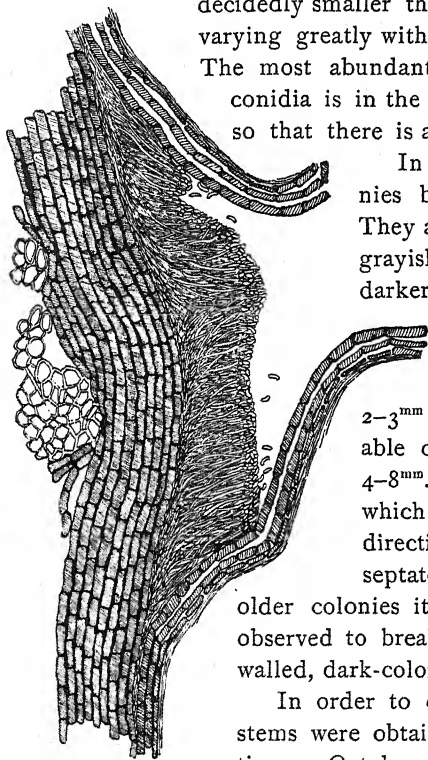


FIG. 8.

In three or four days the stellate colonies become visible to the unaided eye. They are circular in shape, with a slight grayish tinge, elevated and somewhat darker in the center, where the production of secondary conidia is most abundant. In crowded cultures they rarely become more than 2-3<sup>mm</sup> in diameter, but under more favorable conditions may attain a diameter of 4-8<sup>mm</sup>. In its earlier stages the mycelium, which radiates quite uniformly in all directions from the center, is sparingly septate and without vacuoles. In the older colonies it becomes vacuolated, and has been observed to break up into chains of irregular thick-walled, dark-colored cells.

In order to check the results, cultures on bean stems were obtained in various ways and at different times. October 10 two colonies, which were supposed to have developed from the *Gloesporium* spores, were transferred from Petri dish cultures to tubes of sterilized acid bean stems. October 12 the conidia from a single acervulus were teased out in sterilized water, and with the tip of a sterilized needle a very few spores were transferred to each of several tubes of bean stems. October 17 six more colonies were transferred from plate cultures to tubes of bean stems, and four days later four more tubes were inoculated by transferring colonies which had been grown in cell cultures. On the 17th a dilution color was made in acid potato-agar. On the 18th, after the

spores had germinated, a number of them were carefully marked, and on the 21st, when the colonies had become visible to the unaided eye, six of them were carefully transferred to tubes of acid bean stems and three to tubes which had not been made acid. October 25 a similar dilution culture was made, in which on the following day a number of colo-

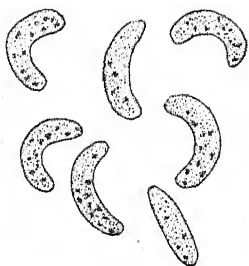


FIG. 9.

nies were carefully marked. These plates were then allowed to stand until November 14 in order that any foreign growths which might be present should have an opportunity to develop, when eight of the marked colonies which still remained entirely distinct from all other growths were transferred to acid bean stems.

The first growth to appear on bean stems is invariably the production of a few scattered, comparatively thick, more or less branched, stromal growths, which arise above the substratum at the point of infection. These shortly become covered with a growth of flocculent white mycelium, and from this center the entire surface of the stem and of the liquid becomes covered with a dense gelatinous looking stroma, which on the surface of the stem is covered with the mycelium (*fig. 4*).

In the course of ten days or two weeks there is usually an abundant production of sori, which are of an olive-green color when seen by transmitted light, but give to the surface of the stroma a glistening black appearance when viewed by reflected light. In the older cultures the stroma has invariably become a deep salmon color, the cause of which has as yet not been determined. We have also observed slight elevations of the stroma which suggested the development of an acervulus, although in no such instances have conidia been observed. In a few instances we have also observed on the surface of the stroma small spherical, olive-green, perithecium-like bodies, covered with a scant mycelial growth, which possibly presage the development of an ascigerous form. In no instance, however, have asci been found in these bodies.

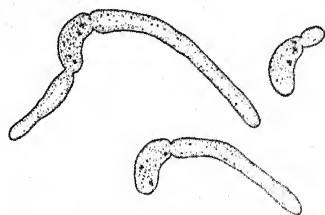


FIG. 10.

To determine whether the fungus studied is the cause of the disease under consideration, on October 30 thirty-six inoculations were

made on sections of apple limbs. Twelve sections, each from 0.75 to 1.50 inches in diameter and 4 to 6 inches long, were selected and divided into groups of four sections each. One of these groups was thoroughly washed in a solution of mercuric chlorid (1 to 1000), after which it was thoroughly rinsed in water. The other two groups were left untreated. The sections in each group were numbered 1, 2, 3, 4, and upon each section three inoculations were made. On sections 1 and 3

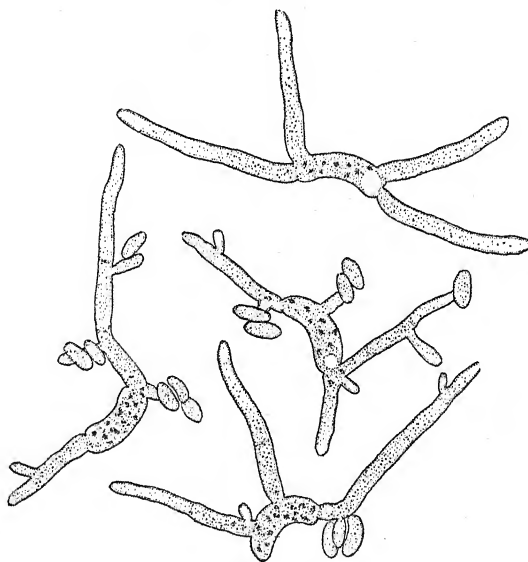


FIG. II.

in each group the inoculations were made by scraping up the epidermis with a flamed scalpel and applying directly to the exposed cortex and cambium small portions of bean stem culture bearing an abundant mycelial growth and conidia. In inoculating sections 2 and 4 care was taken to select portions on which the bark was uninjured and to make the applications without in the slightest abrading the epidermis. The sections when prepared were placed in fruit jars containing moist sand, which had been thoroughly sterilized by steam heat. In about a week after the inoculations were made slightly discolored areas were observed about several of the points of infection, and by November 20, three weeks from the time they were made, these areas had developed all the characteristics of the disease as seen in nature; being



brown, distinctly depressed and separated from the surrounding healthy portions by a zone of hypertrophied tissues which are marked by numerous ragged fissures in the epidermis, through which the underlying chlorophyll-bearing tissues may be seen (*figs. 5, 6, 7*). The peculiar appearance of this zone attracted the attention of Professor Atkinson, who on examination found it to be due to an œdematous condition of the tissues produced no doubt by the excessive supply of moisture in the jars and by the stimulating effect of the fungus. Por-

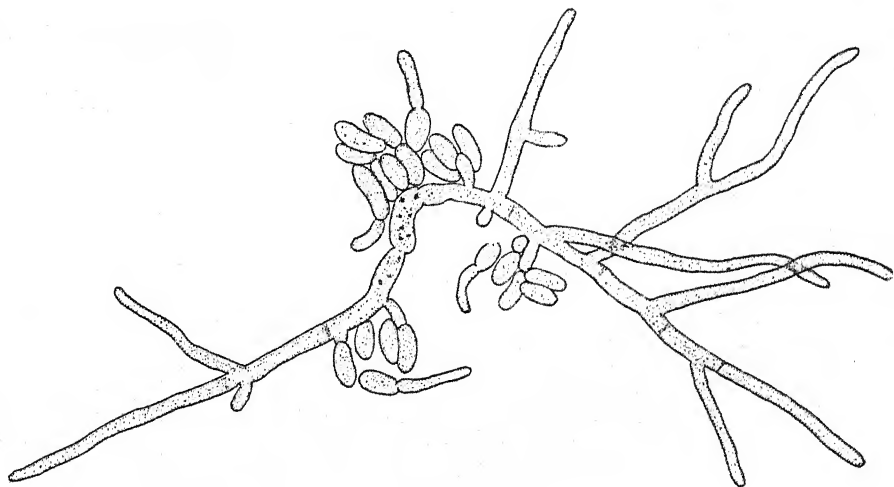


FIG. 12.

tions of these tissues were selected for a more careful histological study, but they were unfortunately lost in an accident and we can only refer for a consideration of œdematous tissues to an article by Professor Atkinson on the œdema of the tomato.<sup>4</sup>

It was hoped that by applying some of the spores to wounded tissues, while care was taken to apply others to uninjured areas of the cuticle, some light might be thrown upon the manner in which the fungus first gains entrance to the cortical tissues; but owing to the fact that no results were obtained from any of the sections treated with mercuric chlorid the test was unsatisfactory.

At the beginning it was realized that such inoculations would not offer absolute proof of the parasitic nature of the fungus, since it is of

<sup>4</sup> ATKINSON, G. F.: Œdema of the tomato. Bull. Cornell Univ. Expt. Sta. 75: 108. *pl. 8*. 1893.

course possible that sections of limbs may offer less resistance to the fungus than they would had they not been removed from the tree. But the fact that these sections were taken from the tree in the fall, and were inoculated immediately before any great change in the tissues could have taken place, together with the fact that the inoculations were followed by such virulent attacks of the disease, seems to offer at least a very strong probability of its parasitic nature.

The fungus, which appears never to have been described, may be characterized as follows:

*Gloeosporium malicorticis*, n. sp.—Parasitic in the cortex of branches of *Pyrus malus*. Affected areas dark brown, limited by ragged irregular fissures, sometimes  $5-7.5 \times 15-20^m$ , occasionally girdling the branch. Acervuli scattered, triangular, rupturing the epidermis,  $300-800 \mu$  in diameter. No pycnidia. Conidial mass at first cream-colored, later dark. Conidia borne on upright basidia, which arise from a subepidermal stroma. Conidia continuous, coarsely granular, at first hyaline, later with greenish tinge, elliptical, curved, sometimes geniculate,  $5-7 \times 16-28 \mu$ , average  $6 \times 24 \mu$ . Those grown in cultures smaller and rarely curved.

To Professor G. F. Atkinson, of Cornell University, I am greatly indebted for advice and assistance.—A. B. CORDLEY, *Oregon Experiment Station*.

NOTE.—Since this article was written, in November 1899, what is probably the same fungus has been described by Professor C. H. Peck in the January number of the *Bulletin of the Torrey Botanical Club*, under the name of *Macrophoma curvispora*. If this supposition proves to be correct the name proposed by me will of course have to be abandoned.—A. B. C.

#### EXPLANATION OF FIGURES.

FIG. 1. *Gloeosporium malicorticis* Cordley; front view of depressed area on apple limb, natural size; photograph taken after the limb had dried.

FIG. 2. Same, side view.

FIG. 3. Front view of old and dead area on a living apple limb, natural size; photograph taken after the limb had dried.

The above three apple limbs were from Oregon, and were photographed by Miss Agnes Vinton Luther in the Botanical Laboratories, Cornell University, during the summer of 1899.

FIG. 4. Fluffy colonies of the pure culture of the fungus in test on bean stems, natural size.

FIGS. 5 and 6. Front view of depressed area as a result of inoculation of pure cultures on sections of living apple limbs at Cornell University.

FIG. 7. Same as *fig. 6*, side view.

Photographs 4-7, by H. Hasselbring, all natural size.

FIG. 8. Section of an acervulus on apple limb showing the basidia and conidia.

FIG. 9. Conidia.

FIG. 10. Conidia germinating.

FIG. 11. Conidia germinating; secondary conidia forming, some of them arising very near the original conidium.

FIG. 12. Germinating conidium with mycelium and new conidia.

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### NEW CARYOPHYLLACEÆ AND CRUCIFERÆ OF THE SIERRA MADRE, CHIHUAHUA, MEXICO.

THE following plants, except the last species (which was collected some years ago upon the Lumholtz Archæological Expedition), belong to an extensive suite (rich in novelties), which is soon to be distributed by Professor E. O. Wooton, of the Agricultural College at Mesilla Park, New Mexico. The collection was made by Messrs. C. H. T. Townsend and C. M. Barber upon a rather arduous journey to a region as yet but little known.

**Cerastium sordidum.**—Densely glandular-tomentose and sordid, 2.2<sup>dm</sup> high: stems thickish, prostrate or nearly so, producing large and slightly fleshy leaf-buds in alternate axils; the foliar leaves, borne chiefly near the ends of these prostrate shoots, oblanceolately acute, 1-nerved, 4<sup>cm</sup> long, 1-1.4<sup>cm</sup> broad, finely and densely tomentose upon both surfaces, persistent and at length glabrate, white and scarious or marcescent: flowering branches erect, subsimple, arising from the thickish buds above mentioned, bearing 2 to 4 pairs of oblong or linear rather small acute leaves below the middle, naked above, terminating in a few-flowered more or less elongated uniparous or irregular cyme: pedicels 1-1.5<sup>cm</sup> long: sepals ovate, herbaceous, sordid-tomentose, 5-6<sup>mm</sup> long, only the acute apex scarious: petals white, 7-8<sup>mm</sup> long, obovate-oblong, somewhat narrowed below the shortly 2-cleft and more or less crisped summit: stamens 5: capsule straight, erect, 1<sup>cm</sup> long; the teeth as in § ORTHODON.—Collected by C. H. T. Townsend and C. M. Barber on the Sierra Madre, 8<sup>km</sup> southeast of Colonia Garcia, Chihuahua, altitude 2310<sup>m</sup>, 30 May, 1899, no. 40. Type in herb. Gray.

**Drymaria Townsendii.**—Delicate glabrous annual: stems filiform, 4-10<sup>cm</sup> long, leafy: leaves slightly fleshy, orbicular, 5-nerved, rounded

at the summit, subcordate at the subsessile base, 4-6<sup>mm</sup> in diameter, about half as long as the internodes, entire; scarious stipules linear-filiform: capillary pedicels spreading, 3-4<sup>mm</sup> long, borne in the upper axils or in short lateral or terminal one-sided bracteate cymes: sepals elliptical, herbaceous except at the white margin, 2<sup>mm</sup> long: petals very narrow, appearing like sterile filaments: fertile stamens 5: stigmas essentially sessile upon the ovoid ovary; seeds pale brown, 0.5<sup>mm</sup> in diameter, muriculate and crested.—Collected by C. H. T. Townsend and C. M. Barber, on the Sierra Madre, 8<sup>km</sup> southeast of Colonia Garcia, Chihuahua, 5 August, 1899, no. 231. Type in herb. Gray.

**Sisymbrium Wootonii.**—Suberect glabrous herb, 5-6<sup>dm</sup> high, branched above; stem glaucous, terete, leafy: leaves obovate-oblong, 5-10<sup>cm</sup> in length, 1.8-3<sup>cm</sup> in breadth, obtusish, cuspidate, entire, thin, somewhat glaucous at least on the lower surface, sessile and auriculate-clasping: pedunculate racemes about 6, erect; pedicels 1-2<sup>cm</sup> long, glabrous, spreading, scarcely thickened at the summit: sepals thin, white, broadly oblong, rounded at the apex, 4-5<sup>mm</sup> long, considerably exceeded by the obovate-cuneate rather broad white petals: young pods 1.4<sup>cm</sup> long, narrowly linear, erect upon spreading pedicels; style 1.5<sup>mm</sup> long; the stigma distinctly 2-lobed, the lobes over the placenta. —Collected by C. H. T. Townsend and C. M. Barber on the Sierra Madre, 16<sup>km</sup> southeast of Colonia Garcia, Chihuahua, 27 July, 1899, no. 176. Type in herb. Gray.

**Sisymbrium microtites.**—Erect, probably biennial, 4<sup>dm</sup> high, simple below, glabrous throughout: radical leaves numerous, pinnately parted nearly or quite to the rhachis; the segments about 9, oblong or elliptical, about 8<sup>mm</sup> long, often with one or more rather salient teeth; cauline leaves 4<sup>cm</sup> long, irregularly bipinnatifid, the terminal segments linear-oblong, exceeding the lateral; petioles provided with small round clasping auricles at the base: racemes elongated; pedicels spreading, filiform, 1<sup>cm</sup> in length, scarcely thickened at the summit: flowers rather small: sepals ovate-oblong, obtuse, pale-yellow, 1.7<sup>mm</sup> long: petals sulphur-yellow, fading white, 2-3<sup>mm</sup> long: pods erect or curved-ascending, 1.4-1.8<sup>cm</sup> long, 2<sup>mm</sup> in diameter, tipped with a short slender style bearing a minute nearly circular stigma; seeds in two rows.—Collected by C. H. T. Townsend and C. M. Barber on the Sierra Madre, 8<sup>km</sup> southeast of Colonia Garcia, Chihuahua, 2 June, 1899, no. 43. Type in herb. Gray. Readily recognized by its minute orbicular ear-like appendages at the slightly broadened base of the petiole.

*Sisymbrium umbrosum*. — Erect branched perennial 6<sup>dm</sup> high; stem pale purple, terete, covered with simple spreading soft white hairs below, nearly or quite glabrous above; slender curved-ascending branches simple: radical leaves several (including the petiole), 1.3–1.5<sup>cm</sup> long; the petiole and midrib densely covered with simple spreading white hairs; the blade lyrate-pinnatifid, appressed-villous on both surfaces, 3.5–4<sup>cm</sup> broad at the widest part; segments rounded and crenately toothed or lobed; cauline leaves much smaller, deeply and rather evenly pinnatifid, short-petioled, exauriculate, the segments 5–9, oblong, acute or acuminate, the sinuses rounded, relatively broad: peduncles 3–10<sup>cm</sup> long; racemes erect, 1.5–3<sup>dm</sup> long; pedicels widely spreading or ascending, sparingly pilose or glabrate, slightly thickened at the summit: sepals ovate-oblong, obtuse, 3<sup>mm</sup> in length, sometimes villous dorsally: petals white, rather narrow, 6<sup>mm</sup> long: pods very narrow, linear, about 4<sup>cm</sup> long, loosely spreading, often curved outward, glabrous, 0.7<sup>mm</sup> broad, scarcely beaked; the stigma obscurely 2-lobed; the lobes over the placentæ; seeds apparently in 1 row. — Collected by C. V. Hartman in shady places among rocks, Puerto de San Diego, Chihuahua, altitude 2000<sup>m</sup>, 12 April, 1891, no. 629. Type in herb. Gray. Distributed as *Thelypodium auriculatum*, of which it has somewhat the habit; from this, however, it differs markedly in pubescence and exauriculate leaves. The orientation of the stigma places this species in *Sisymbrium* rather than *Thelypodium*. — B. L. ROBINSON, *Gray Herbarium*.

## CURRENT LITERATURE.

### BOOK REVIEWS.

#### The Flora of Montana.<sup>1</sup>

NOT since Dr. Coulter's *Manual of the Flora of the Rocky Mountains* was issued has so important a contribution to the botany of this region appeared as Dr. Rydberg's work which now lies before us. The workers in this vast empire, known as the Rocky mountains, learned with pleasure, some months since, that this author was engaged upon a *Flora of Montana*, and its appearance was awaited with some impatience. The magnitude of the task was probably only half appreciated even by those who are actively engaged in the study of the plants of this same general region. Though the title-page denominates it a *catalogue* of the flora, it is far more than that, and is likewise more than an annotated catalogue. Each species is treated almost exhaustively: citations of publication, the more important synonymy, notes upon habitat and distribution are given, and following this an enumeration of the specimens examined. In the case of new species, specimens from adjacent states are also cited, in so far as they were known to the author.

The introduction is interesting in showing the circumstances which led to the inception of the enterprise, with some history of the exploring expeditions which secured, in part, the collections upon which the *Flora* is based. Nearly all the collections that have been made from the time of Lewis and Clark to the present seem to have been available for study, and it is really remarkable how many expeditions have touched to a greater or less extent upon Montana soil.

In the development of the botany of western America it is noticeable that the early explorations were, in large part, in the far west or northwest; that even the arctic portions of British America were known long before the interior west of the United States. The names now so familiar to us as collectors and explorers, Drummond, Douglas, etc., are embalmed in the literature which has been the basis of west American botany. In the Rocky mountain region of the United States the southern half was developed earlier than the northern, as witness the work of Fendler, of the Mexican Boundary Survey, of the Wheeler Expedition, etc. In the northern half, we have the Pacific Railroad Surveys, King's Expedition, Parry's and Coulter's Reports, etc., but even these dealt largely with the plants of the Pacific slope

<sup>1</sup>RYDBERG, PER AXEL: Catalogue of the Flora of Montana and Yellowstone National Park. Memoirs of the New York Botanical Garden. Vol. I. 8vo. pp. xi + 492.

rather than those of the eastern slopes of the Rocky mountain range. Nuttall, it is true, must have collected over a very large area of this interior west, but what is one man's work in such a vast region, even though he be a Nuttall? Keen in observation, discriminating in description, Nuttall stands without a peer as regards the field in question. For sixty years following his time some of the regions that he visited have not been entered by other botanists. On account of the inadequate specimens that he made, and their inaccessibility to the majority of workers, many of his species were for years rather discredited, or at least misunderstood. With the renewed interest that has sprung up during the last decade, Nuttallian species are again at par. Furthermore, new ones are being described by the score every year. From a long period of conservatism, during which the plants of the interior west were often disposed of as mere forms of species of widely different geographical range, we have now come to look upon this flora as quite as sharply defined as that of any other area of equal size. In the work before us we have an excellent example of this tendency.

The remarkable number of 1976 species and varieties are reported in Dr. Rydberg's *Flora*, though only the Spermatophytes and Pteridophytes are included. Of these the author indicates 776 as not found in Coulter's *Manual*, including 163 which are described as new. A few species have been admitted simply on report, but the author aimed to admit only those which rest upon good authority. In spite of this excellent showing as to numbers, the author assures us that large sections of the state are still almost a *terra incognita*. Should these portions of the state yield in the same ratio, and the other Rocky mountain states prove equally prolific, the future Manual of the Rocky mountains will be a work of no mean magnitude.

Altogether the work is so admirably done, and contains so much of value to those who are interested in the taxonomy of western seed-plants, that one might be justified in wholly overlooking any inaccuracies, and in avoiding all discussion of those conclusions which rest, in a measure at least, upon individual judgment. But it may not be out of place to call attention to some matters that the author desired otherwise as much as anyone, and to some of the points raised which may be clarified by discussion.

The large number of species reported would have been materially reduced had the work been done by one more conservative. What constitutes a specific difference is still one of the unanswered questions, and is likely to remain so for sometime, but it is easily discoverable that to the describer with the plant in hand the differences appear more fundamental than they do to one who has to perceive them solely through the description. Dr. Rydberg's treatment illustrates this. In Dodecatheon he lists ten species, describing five as new, some of which seem to the reviewer to be based upon inadequate differences and insufficient material. For instance, of *D. pulchrum* it is stated "it agrees with the description of *D. conjugens* Greene except

that the petioles of the leaves are obsolete," and then he finds the species on Mr. Tweedy's no. 432, in part, the other part being cited under *D. cylindrocarpum*, another new species which is also said to be nearly allied to *D. conjugens*. The familiar *Arnica alpina* Olin (or more properly *A. fulgens* Pursh) has come in for its share of segregation. Three or four species are recognized, but the writer is unable to discriminate between them, and somehow has the feeling that here we have had a splitting of hairs. Likewise *Phyllodoce* (*Bryanthus*) has had critical attention, four species being recognized, though it is suggested that two of them may be hybrids. Of these four species two were collected by the writer last summer (*P. empetritiformis* and *P. intermedia*), both growing in the same little patch in an alpine gulch, and no difference was detected except that of color. Other genera might be mentioned, but one need not cite further examples.

Somewhat in contrast with this is the occasional accrediting to Montana of species that are supposed to belong to quite a different geographical range. *Erigeron speciosus*, *Claytonia Virginica*, and *Phlox Douglasii* may serve as examples. The following names occur in the first seventeen pages of the text, though no specimens are cited: *Dryopteris Filix-Mas*, *Pinus scopulorum*, *Abies amabilis*, *Juniperus occidentalis*, *Potamogeton natans*, and *P. Robbinsii*. It is hardly necessary to state in this connection that the work does not seem to be even; some genera have received the most painstaking treatment, while others have been handled in a less critical manner. This, however, could scarcely be otherwise in a work of such magnitude.

It may be interesting to note that there are discoid forms in *Erigeron trifidus* as well as in *E. multifidus*, and that if these are to be recognized as a variety in one we must do so in the other. It would not be surprising to find that *E. compositus* Pursh also has its discoid forms.

Much obscurity in the past as to the limits and identity of species has been caused by inadequate descriptions. So here the descriptions, in some instances, seem likely to give rise to similar confusion, not always because of their brevity, but rather because of the omission of essential characters. In *Carduus canovirens* very characteristic pubescence of two kinds is passed over as "more or less woolly." In *Artemisia tenuis* we have no mention of the flowers as to number, form, size, or kind, nor of the akenes, though to this species is appended a variety, "growing with the type," to which less than two lines is devoted. However, authors will of necessity differ as to what are essentials.

*Washingtonia divaricata* (Nutt.) Britt. and *Phlox andicola* (Nutt.) Britt. are examples of a practice in which the author is not in accord with most other writers. Nuttall did not describe these species, and though he had used the mere names he should not be cited as if he were the author. Dr. Britton in the *Illustrated Flora* very properly did not do so.



A matter of punctuation: In harmony with the general practice at present, Dr. Rydberg writes *Mimulus Lewis* Pursh, Fl. Am. Sept., but writes *Monniera rotundifolia* Michx. Fl. Bor. Am. The query in my mind is, if we use a comma to separate the author's name from the name of the publication in one case, why not in the other? The period is a part of the abbreviated name and does not set it off from the name of the publication. Why not write *M. rotundifolia* Michx., Fl. Bor. Am.?

In most instances the approximate altitudinal range for the species is given, a practice to be commended, though such statements must always be allowed some additional variation, since so much depends upon the exposure and general conformation of the country. Especially is this true where but few collections are at hand upon which to base the statement. On this account it is sometimes a little amusing to note that a definite range is sometimes given when but a single collection was known, as for instance in *Atriplex hastata*, *Grayia spinosa*, *Abronia fragrans*, *Washingtonia longistylis*, etc.

As mere slips, typographical or otherwise, may be noted the following: On p. 130 Shoshone lake is accredited to Montana; on p. 184 the notes speak of *Sophia canescens*, though I think the *Sisymbrium canescens* Nutt. has never been transferred to that genus; on p. 417 *Balsamorhiza sagittata* Nutt. should be *B. sagittata* (Pursh) Nutt.; on p. 377 the *not Pursh* following *Valeriana pauciflora* Hook. should read *not Michx.*; on p. 386 the *Solidago multiradiata* listed is more probably *S. multiradiata scopulorum*. It may be noted, too, that in some instances the notes upon a species are not in accord with the name. While the *Flora* was going through the press it became necessary to change from the original name selected, and the notes were not altered to correspond. In one or two instances A. Nelson is credited with a species that belongs to E. Nelson, e. g., *Pellea occidentalis*, p. 466.

Of course the author uses the Engler and Prantl arrangement which can scarcely fail of universal approval. The application of the Rochester rules has resulted in the displacement of some of the familiar names, and even to one who thoroughly believes in the principle of priority it is a source of regret that it should necessitate so many changes.

All errors aside, the volume before us, with its nearly five hundred octavo pages, will prove invaluable to the working botanist, and will also prove of interest and service to the amateur, though it is not put out as a manual of the territory covered. It reflects great credit upon the author, and is an honor to the New York Botanical Garden from which it is issued as Volume I of its *Memoirs*. The excellent paper, luxurious type, and broad margins, make it a pleasure to the eye.—AVEN NELSON.

### The Cell.

THE first edition of Professor Wilson's work<sup>2</sup> on the cell, which appeared in 1896, met a hearty welcome from biologists everywhere; it was reprinted the next year with the addition of an appendix which summarized the recent literature, and was again reprinted in 1898 with a similar appendix, but the literature of the subject had increased so much and the aspects of important problems were changing so rapidly that something more than an appendix was needed to bring the book up to date. Accordingly the entire work has been thoroughly revised, and over one hundred pages and fifty figures have been added. The problems of the centrosome, cell division, and fertilization, have been materially changed, some of the sections having been entirely rewritten. Recent experimental work in these subjects and in regeneration have received special attention. Changes and additions, however, are not confined to these subjects, but are found upon almost every page.

The treatment, as in the previous edition, is historical, and the careful citation of specific authority which enables the reader to weigh for himself the statements of the text makes the book indispensable to cytologists, whether they be zoologists or botanists.

The book, though still confessedly weak in the field of botanical cytology, shows a decided improvement over the previous edition, especially in the subjects of cell division, spermatogenesis, and fertilization. It is helpful to the botanist to get a view of his own problems from a zoological standpoint.

It is of special interest to botanists to note that the author regards the blepharoplasts of *Gingko*, *Cycas*, *Zamia*, *Marsilea*, etc., as genuine centrosomes, although he believes that Webber and Ikeno have produced apparently strong evidence that they arise separately and *de novo* in the cytoplasm.

He hesitates to accept Shaw's statement that in *Marsilea* the "blepharoplastoids" have no relation to the blepharoplasts which appear later; a decision is also withheld in regard to Webber's conclusion that in *Zamia* the blepharoplasts have a separate and independent origin. In regard to vegetative mitoses of the higher plants the statement is made that we should still hold open the possibility that centrosomes may occur, their apparent absence being possibly due to lack of staining capacity or similar conditions rendering their demonstration difficult.

"In the female the two maturation divisions give rise to the four primary cells of the embryo sac: and these two divisions undoubtedly correspond to the two maturation divisions in animals. In the female only one of the resulting cells gives rise to the egg, the other three corresponding to the

<sup>2</sup> WILSON, E. B.: The cell in development and inheritance. 2d edition, revised and enlarged. 8vo., pp. xxi + 483, *figs.* 197. New York: Macmillan Company. 1900. \$3.50.

polar bodies of the animal egg, though they here continue to divide and give rise to rudimentary prothallium."

Morphologists of today can hardly accept the statement that the "pollen tube represents a rudimentary male prothallium or sexual generation."

The paragraph on fertilization in plants has been considerably improved, notably by the account of double fertilization in angiosperms.

Considerable space is devoted to the reduction of the chromosome in plants, but no decision is reached as to whether there is a reducing division such as has been described by vom Rath and others.—C. J. CHAMBERLAIN.

### MINOR NOTICES.

A SECOND EDITION of Wiesner's *Die Rohstoffe des Pflanzenreiches* has begun to appear from the press of Wilhelm Engelmann.<sup>3</sup> Three parts, each containing 160 pages, have already been published, and the remaining two parts of the first volume are promised before the close of the present year. Another volume of the same size will be needed to complete the work, and probably its publication will be well advanced within the year.

As compared with the first edition, issued in 1873, the present is completely rewritten and extended to about double its former size. In the present parts Professor Wiesner writes the introduction (47 pp.) and the sections on gums (82 pp.), and resins (226 pp.); Professor K. Mikosch writes on the caoutchouc group (43 pp.), the catechu group (14 pp.), and the fats (incomplete); Professor A. E. Vogl on opium (14 pp.) and aloes (10 pp.); and Professor H. Molisch on indigo (24 pp.).

This collaboration (the complete list of collaborators, as announced, includes eleven names) puts the different subjects into the hands of specialists and insures prompt completion of the work.

In each section, varied to suit the special case, the general plan of treatment includes an account of the plants yielding the substance and their distribution, the mode of obtaining it, its commercial characteristics, its physical, chemical, and microscopical qualities, and its uses. Both botanical and technical users, therefore, will find this work a mine of information regarding plant products.—C. R. B.

THE ELEVENTH EDITION of Prantl's *Lehrbuch der Botanik*<sup>4</sup> has been issued by Engelmann. The revisions of this popular text-book have been under the charge of Dr. Pax, of Breslau, since the death of the author.

<sup>3</sup> WIESNER, JULIUS: *Die Rohstoffe des Pflanzenreiches. Versuch einer technischen Rohstofflehre des Pflanzenreiches. Zweite gänzlich umgearbeitete und erweiterte Auflage.* Leipzig: Wilhelm Engelmann. 1900. Pro. 10. M 5.

<sup>4</sup> PAX, FERDINAND: *Prantl's Lehrbuch der Botanik. Elfte verbesserte und vermehrte Auflage.* 8vo, pp. viii + 455, figs. 414. Leipzig: Wilhelm Engelmann. 1900. M 4.60; geb. 6.10.

Without changing the plan of the book its statements have been changed to conform to recent discoveries. But a more thorough change, especially to strengthen the physiological section (of only 50 pp.), seems desirable. The main emphasis is laid on the third part, the taxonomic synopsis of the plant kingdom, nearly half the book being devoted to descriptions of families and orders of phanerogams. There must be a demand for this sort of thing in Germany, especially in the schools of pharmacy, else there would not be so many text books in which it is prominent. But it is hard to see of what possible didactic value it can be, and the space would be better used if devoted to a thorough discussion of the fundamentals of morphology and classification.—C. R. B.

THE FIRST FASCICLE of the list of the genera of seed plants, according to the system of Engler, has appeared.<sup>5</sup> The large page is divided into two columns, and all of the divisions down to the genera are given. The genera are numbered in double sequence, the first number indicating the place in the whole series of genera, the second number indicating the place in the family. In these first signatures 1268 genera are listed, beginning with *Cycas* and closing with *Marica* (Iridaceæ). Under each genus there are given the important citations, the synonymy, the approximate range and number of species. In the case of larger genera the section names and their synonymy are given. This work will not only be indispensable in herbaria, but will be a mine of handy information to the general student of morphology and taxonomy.—J. M. C.

A NEW EDITION of the Bonn text book,<sup>6</sup> the fourth, has been issued by Fischer, with the purpose of keeping this incomparable work abreast of the science of botany. The authors have incorporated from the newer literature everything which appears to them to be of scientific or didactic value. Certain sections of the part on external morphology have been extended, some new illustrations have been added and some old ones replaced by better. The general plan of the book is not altered. It has become so well known that there is no need to speak of its merits or defects. We hope that the translator of the English edition will take advantage of the new German edition to bring the English one also up to date and correct some slips of translation.—C. R. B.

<sup>5</sup>DALLA TORRE, C. G. DE, and HARMS, H.: Genera Siphonogamarum ad systema Englerianum conscripta. Fasciculus primus (signatura 1-10). Small 4to, pp. 80. Leipzig: Wilhelm Engelmann. 1900. *M* 4.

<sup>6</sup>STRASBURGER, NOLL, SCHENCK, and SCHIMPER: Lehrbuch der Botanik für Hochschulen. Vierte verbesserte Auflage. 8vo, pp. viii + 588, *figs.* 667. Jena: Gustav Fischer. 1900. *M* 7.50.

## NOTES FOR STUDENTS.

IN SEARCH of an explanation for mycorrhiza Dr. E. Stahl has made a comparative study of broader scope than any involved in the previous researches upon the subject.<sup>7</sup> The interesting conclusion of extended observations upon the distribution of mycorrhiza is that, hydrophytes aside, the large majority of vascular plants possess it. Its occurrence, in the words of the author, "has an intimate relation with increased difficulties of nutrition."

That photosynthesis is so intimately conditioned by transpiration as to be approximately in direct proportion to it is a fundamental premise of Stahl's conception. Further, it is assumed as demonstrated that the host, in the presence of mycorrhiza, possesses a nutritive advantage. Now if it be found that mycorrhiza is coincident with low transpiration, may not the nutritive advantage of the former provide a certain compensation for the weakness of the latter? Indeed the author seeks to refer the inception of mycorrhiza to comparative weakness of absorption as an original cause. For, he argues, a root system unable to supply the green parts with a transpiration stream carrying nutrient salts commensurate with the demand would at once induce a need for equivalence of organic substances which it is the function of the mycorrhiza habit to supply. Holding with Frank that the humus soil of forests is in large part "a living mass of innumerable fungal hyphæ," the discussion of competition for nutrient salts upon forest floors leads to the conclusion that, unless possessed of root-systems of exceptional absorbent efficiency, the vascular green plant is placed at decided disadvantage; this notwithstanding the great and apparently available store of nutritive material, and on account of the ubiquity and chemotropic advantage of the rival fungal hyphæ. The obligate mycorrhiza plants with low transpiration cannot, it is argued, independently compete for nutrient salts with vascular plants of high transpiration or with the fungi, but they escape the stress of that competition by making tributary to themselves certain fungi from which they supplement the supply of elaborated organic compounds. That holosaprophytism involves no more than increased reliance upon the fungal tributaries and corresponding loss of even potential photosynthesis is obvious. Stahl attributes the inception of the parasitic and carnivorous habits to the same handicap in competition for the nutrient salts. There should be noted herein a certain parallelism of argument with the claim of Frank for definition of endotrophic mycorrhiza as "fungus-traps," although Stahl does not agree with Frank in holding to a different physiological significance between the ectotrophic and endotrophic forms.

It is quite unlikely that the author will escape criticism for the breadth of certain of his generalizations or for his use of certain facts as positive

<sup>7</sup>STAHL, E.: Der Sinn der Mycorrhizenbildung. Jahrb. f. wiss. Bot. 34: 539-668. 1900.

criteria when an interpretation totally at variance from that upon which his conclusions depend is at least admissible. Thus at the very outset he devotes a number of pages to the discussion of "criteria for the relative water-demand of plants." He seeks a ready means of determining in extended observations whether or not mycorrhiza when present is associated with reduced transpiration, as his hypothesis demands. The presence of starch rather than sugar in the leaves is selected as the foremost characteristic for his purposes indicative of greater transpiratory activity. "For," he writes, "the advantage of starch formation lies very largely in the promotion thereby of transpiration, since reduced concentration stimulates the evaporation rate; and, inversely, an increase of the dissolved substances, glucose, for example, must have a retarding effect." But it is a fair question whether starch may not appear only after a maximum concentration has been attained, and hence its absence regarded as favorable rather than opposed to transpiration.

Certain experiments upon *Sinapis alba*, *Linum usitatissimum*, and *Triticum vulgare*, forms free of any trace of mycorrhiza, are of interest in affording striking contrast with Frank's experiments upon beech and pine seedlings. In the latter case, as is well known, the development in a sterilized soil of seedlings normally possessed of mycorrhiza was strikingly deficient in contrast with individual plants in unaltered humus soil. But, if the contention of Stahl is sustained, autotrophic forms should flourish in especial degree in soil where they are freed of the rivalry of fungi, which unlike their more specialized mycotrophic kin, they are unable to make tributary; *i. e.*, exactly the reverse of the results with mycotrophic forms. And such is found to be the case in striking degree. Incidentally, however, an excursion is made to demonstrate the retarding effect of abundance of nutrient salts upon root growth. For, though the shoot is feeble, the root system, as is reasonable to expect, becomes more elongated in the autotrophic forms grown in competition with the fungi.

That part of the paper which deals with "the absorption of nutrient salts and the ash-content of mycotrophic as compared with autotrophic plants" is none the less important in that it seems to belong as a closing chapter to such special studies of this subject as those of MacDougal and Groom rather than to a contribution so broadly comparative in character as this one. The introductory argument that the ash content of mycotrophic plants should have certain specific differences from that of autotrophic forms is more safely founded upon the previous and detailed study of others, than upon a general association of mycorrhiza with forms limited in their possibilities of independent food-elaboration, however broad and striking that association may be. Suffice it for the argument that his examples show a certain parallelism to exist between a greater ash-content and autotrophy on the one hand, and less ash-content and mycotrophy on the other. This tends to

confirm the idea that mycotrophic plants receive from the fungi certain necessary mineral substances already in the form of organized compounds, for thereby they would be relieved to that extent of the necessity, which devolves upon all wholly independent forms, for receiving into the plant body a certain mineral surplus, of which calcium, received as the sulfate, nitrate, or phosphate, is a conspicuous example.

Whatever the disappointment may be that by this contribution, the question of distribution aside, we are no nearer than before to positive knowledge of the nature of this remarkable symbiosis, it is the presentation of an admirable hypothesis of the general function and distribution of mycorrhiza, supported by a wealth of evidence which subsequent studies are far from likely to controvert. To the practical physiological ecologist the lists and detailed discussion of mycorrhiza distribution will be found invaluable.

New data as to the mycorrhizas of holosaprophytes and the structural degeneracy attendant upon this symbiosis are presented by MacDougal and Lloyd.<sup>8</sup> Species of *Pterospora*, *Sarcodes*, *Hypopitys*, and *Monotropa* were examined. Special emphasis is laid upon the profound morphological alterations of the host which ensue from such complete symbiosis as that which exists between the *Monotropaceæ* and the ectotrophic fungi of its roots. In this family the shoots are to be regarded as physiologically insignificant in the economy of the plant, being purely reproductive in purpose and comparatively ineffective in that function. The roots, which reproduce the plant, serve as organs of storage, are the habitat of the fungus, the absorbing member of the symbiosis, and are the seat of the chief activities of the plant. *Pterospora* shows an interesting exception to the general tendency of shoot reduction in its development of a comparatively large branched aerial portion which is supplied with stomata.—J. G. COULTER.

ITEMS OF TAXONOMIC interest are as follows: In *Proc. Biol. Soc. Washington* (13: 129-132. 1900) C. L. POLLARD has published eight new species, belonging to *Lupinus*, *Viola* (2), *Gentiana* (3), *Chrysopsis*, and *Solidago*.—The species and hybrids of *Mentha*, a paper by ERNEST MALINVAUD, has been translated into English and published in *Journ. Bot.* 38: 171-174. 1900.—A list of the angiosperms of Delagoa Bay, Africa, has just been published by HANS SCHINZ (Zürich) and Henri Junod (Delagoa Bay) in *Mém. de l'Herb. Boiss.* (no. 10. March 30, 1900.) No. 11 of the same series (issued April 30) is devoted to a continuation of *Species Hepaticarum*, by FRANZ STEPHANI, the genera *Hymenophyllum* (4 spp.), *Pallavicinius* (22 spp., 5 new), *Symphyogyna* (39 spp., 14 new), and *Monoclea* (2 spp.) being included. No. 12 of the same series (issued April 30) contains an account (with 4 plates)\* of the European species of *Utricularia*, by FR. MEISTER, together with a

<sup>8</sup>MACDOUGAL, D. T., and LLOYD, E. F.: The roots and mycorrhizas of some of the *Monotropaceæ*: Bull. N. Y. Bot. Garden 1: 419-428. 1900.

discussion of their anatomical and ecological features. No. 14 of the same series (issued May 30) contains an account (with 1 plate) of the Japanese Mutisiaceæ from the collection of Faurie, by A. FRANCHET. No. 15 of the series (issued May 30) contains an account of certain new or little known Chytridineæ, by E. DE WILDEMAN.—Notes on some type specimens of Myxomycetes in the New York State Museum is a useful paper by W. C. STURGIS, published in *Trans. Conn. Acad. Arts and Sci.* 10: 463-490, pls. 60, 61. 1900.—F. E. LLOYD and L. M. UNDERWOOD (*Bull. Torr. Bot. Club.* 27: 147-168. pls. 2-4. 1900) have published a review of the North American species of *Lycopodium*, recognizing 29 species, two of which are described as new.—P. A. RYDBERG (*ibid.* 169-189. pls. 5, 6) has begun a series of papers entitled "Studies on the Rocky Mountain Flora," the first one being devoted to the *lobatus*, *aureus*, *subnudus*, and *tomentosus* groups of *Senecio*, in which assemblage of forms he recognizes 30 species, 18 of which are described as new.—W. R. MAXON (*ibid.* 197-199) has described a new *Asplenium* from southern and Lower California.—S. C. STUNTZ (*ibid.* 202-211) has published a revision of the N. Am. species of the genus *Eleutera* (Neckera), recognizing 6 species, all with new names.—G. N. BEST (*ibid.* 221-236. pls. 7, 8) has published a revision of the N. Am. species of *Pseudoleskea*, recognizing 7 species, two of which are new, and 4 varieties, all of which are new.—E. P. BICKNELL (*ibid.* 237-246) has reached his seventh paper on *Sisyrinchium*, which deals with the species of British America. He recognizes 8 species, one of which is described as new, and there is no reason to question his statement that "there is no doubt that this number may be increased."—W. W. ROWLEE (*ibid.* 247-257. pl. 9) has published an account of the *longifolia* group of willows as displayed in North America. He recognizes 12 species, three of which are described as new, and 3 varieties, all of which are new.—AVEN NELSON (*ibid.* 258-274), in continuing his account of new plants from Wyoming, has described 23 new species and varieties.—J. K. SMALL (*ibid.* 275-281), in continuing his notes and descriptions of N. Am. plants, has described 11 new species.—FLORA W. PATTERSON (*ibid.* 282-286) has described 17 new species of fungi.—GEO. V. NASH (*Bull. N. Y. Bot. Garden*, 1: 429-437. 1900) has described 11 new grasses from the southern states, and a new *Trisetum* from Michigan.—J. K. SMALL (*ibid.* 437-447) has published a revision of the genus *Bumelia* in North America, recognizing 13 species, five of which are described as new.—N. L. BRITTON (*ibid.* 447-449) has described 7 new species of *Crataegus*.—MRS. KATHARINE BRANDEGEE has published a second paper in the series on Cactææ (*Zoe* 5: 1-9. 1900), the first having appeared in *Erythea* (3: 123. 1895). It contains critical remarks upon numerous species, and descriptions of one new species of *Cereus* and two of *Mamillaria*.—J. M. C.



## NEWS.

PROFESSOR DR. H. AMBRONN has been called to the assistant professorship of botany in the University of Jena.

PROFESSOR J. W. TOUMEY, of the University of Arizona, has been appointed assistant professor of forestry in the new school of forestry at Yale University.

WITH THE BEGINNING of the present volume of the *BOTANICAL GAZETTE*, Dr. J. C. Arthur, on account of ill-health and pressure of work, retires from the position of an active and responsible editor. For eighteen years he has been identified with the journal, and has been a large factor in whatever success it has achieved. In 1882 he became an associate editor, and in 1886 he became an editor, sharing financial as well as editorial responsibilities. The *GAZETTE* will not lose his counsel and active cooperation, for his connection will be continued as an associate editor.

THE FOLLOWING FELLOWS in Botany have been appointed by the University of Chicago for 1900-1901: S. E. M. Coulter (Hanover College), B. E. Livingston (University of Michigan), and A. A. Lawson (University of California). A. C. Moore, a Fellow of last year, has been appointed Assistant. Mr. J. E. Webb, who received the Master's degree in June, takes charge of the work in botany at Morgan Park Academy, a preparatory school of the University. Mr. F. L. Stevens, who received the Doctor's degree in June, is to spend two years abroad in botanical work as an honorary fellow.

WE LEARN from *Science* (June 15) that the appropriations for the Department of Agriculture for the fiscal year ending June 30, 1901, amount to \$4,023,500, being an increase of more than \$280,000 over the appropriations for the preceding year. Of this amount \$720,000 is for the experiment stations in the forty-eight states and territories, \$12,000 for the stations in Alaska, \$10,000 for a new station in Hawaii, and \$5000 for an investigation and report to Congress on the agricultural resources and capabilities of Puerto Rico. The appropriations which directly concern the botanical establishments are as follows: Division of Botany, \$43,080; Division of Forestry, \$40,000; Division of Vegetable Physiology and Pathology, \$34,500; Division of Agrostology, \$25,100. The Biological Survey receives an appropriation of \$30,300; while the appropriation for the Division of Seeds is increased from \$130,000 to \$170,000, an increase due in large measure to a petition of some 225 members of the House of Representatives.

# BOTANICAL GAZETTE

AUGUST, 1900

## THE DEVELOPMENT AND FUNCTION OF THE CELL PLATE IN HIGHER PLANTS.

H. G. TIMBERLAKE.

(WITH PLATES VIII AND IX)

### Historical.

ASIDE from the work of Strasburger there have been very few investigations reported that have treated fully the subject of the cell plate and its history in the vegetable cell. So far as I know, the term cell plate was first used in its present sense by Strasburger in the first edition of *Zellbildung und Zelltheilung*. It was here that he made the statement that the beginning of the cell plate is to be found in swellings of the connecting spindle fibers. The subject was more fully discussed in the third edition of the above work, and I shall refer to that in greater detail below.

In 1878 Treub published his classic researches on the rôle of the nucleus in cell division,<sup>1</sup> in which he describes the process of cell plate and cell wall formation in the living cells of the proembryo of *Orchis latifolia* and the ovules of *Epipactis palustris*. By keeping the tissues in a 1.25 per cent. solution of  $\text{KNO}_3$  he was able to make an extended study of the above processes. The first indication of the formation of a cell plate is a collection of granules across the equatorial region of the spindle, which move into place from various directions in the cytoplasm. They

<sup>1</sup> Quelques recherches sur le rôle du noyau dans la division des cellules végétales. Amsterdam, 1878.

have, therefore, no connection with the spindle fibers. After reaching the equator the granules fuse into a continuous layer across the central spindle. The growth of the cell plate occurs by the addition of new material either all around, if the spindle is in the middle part of the cell, or on the free sides, if the spindle is near one side of the cell so that the cell plate has reached the mother cell wall on that side where it is first formed, *i. e.*, the whole nuclear figure moves across the cell, building the cell plate as it goes. By plasmolyzing the cell by the addition of more  $\text{KNO}_3$  Treub was able to demonstrate that the new cell wall is laid down between the split halves of the cell plate.

In the third edition of *Zellbildung und Zelltheilung* Strasburger gave the most comprehensive account of the cell plate that has ever appeared. One of the important facts that he describes in this work is the apparent increase in the number of connecting fibers prior to the formation of the cell plate (*op. cit.*, p. 341). There are a few spindle fibers extending through the cell plasma between the receding groups of daughter chromosomes. These fibers can be distinguished from the surrounding cell plasma by their filar form. Their number is increased by the addition of new fibers differentiated out of the cytoplasm. The new fibers are identical, in so far as their staining qualities and form are concerned, with the original fibers. This apparent identity is evidence for the cytoplasmic origin of spindle fibers. The hypothesis is strengthened by the process of division of those pollen mother cells which divide simultaneously, where new connecting fibers are formed from the cytoplasm after the two daughter nuclei of the first division have again divided, and the original connecting fibers, with the cell plate formed in them, have disappeared. The permanent cell plate is then formed in these new fibers (*op. cit.*, p. 345). In the spore mother cells of *Anthoceros* and the macrospore mother cells of *Isoetes*, the spindle fibers in which the cell plates are formed connect the four daughter plasma masses or chromatophores, instead of the nuclei. Davis<sup>2</sup> has recently reinvestigated the division of the

<sup>2</sup> The spore mother cell of *Anthoceros*. BOT. GAZ. 28: 89. 1899.

spore mother cell of *Anthoceros* and has found that the cell plate is formed upon cytoplasmic strands (not spindle fibers) uniting the four chromatophores. A portion of this cell plate becomes converted into a cell wall.

In endosperm formation there are a great number of nuclear divisions that are not followed directly by divisions of the cell. The spindle in each case disappears after each nucleus divides.<sup>3</sup> When the above process has continued until there are a great many nuclei lying free in the protoplasm, new fibers are formed connecting each nucleus with all of its immediate neighbors. In these fibers the cell plates are built in the usual manner. In a later work<sup>4</sup> these new fibers were described as growing out of a hyaline plasma mass around each nucleus.

On the nature of the cell plate elements, *i. e.*, the granules which form the cell plate, we have an interesting statement.<sup>5</sup> The cell plate is formed of small granules whose chemical nature is hard to determine. That they may be starch is indicated by the fact that they take in some cases a blue stain when treated with iodine. In most cases, however, they are not thus stained. They may be a substance between starch and cellulose. This hypothesis is rendered more probable by the fact that the cell plate elements are apparently used directly to form a cellulose wall instead of being converted into a protoplasmic layer which splits and excretes a cellulose layer between the halves.

In the pollen mother cells of the *Cycadaceæ*, Juranyi<sup>6</sup> described a process of cell wall formation by the conjunction of a cellulose ring, growing in from the mother cell wall and a new wall formed in the connecting spindle.

In 1882 Strasburger modified his previous view as to the origin and chemical nature of the cell plate elements.<sup>7</sup> In this paper he holds that with the aid of suitable stains it can be shown

<sup>3</sup> STRASBURGER, *op. cit.*, p. 345.

<sup>4</sup> Ueber Kern- und Zelltheilung im Pflanzenreich. Hist. Beitr. I. 1888.

<sup>5</sup> Zellbildung und Zelltheilung, 3. Aufl., 342.

<sup>6</sup> Mittheilungen über Structur u. Bildung des Zellkernes. Ref. in Bot. Centralb. 12: 213. 1882.

<sup>7</sup> Ueber den Bau u. d. Wachsthum der Zellhäute 172. Jena, 1882.

that the granules of the cell plate react as albumen. As to the origin of these granules it was observed that they are not added from the surrounding protoplasm but are within the fibers themselves. The fibers appear as if made up of small granules. These granules are really contained in the fibers as if in tubes, and they collect at the equator, forming apparent equatorial swellings of the fibers, *i. e.*, the cell plate elements. The cell plate elements, after growing by the addition of new granules, finally fuse into a continuous plate.

In 1887 Went<sup>8</sup> showed that connecting fibers and other spindle fibers are identical, and that the former apparently increase in number before the cell plate appears. He was not able, however, to account for the origin of the new fibers. Prior to the formation of the cell plate, Went observed a stained substance between the fibers of the connecting spindle next to the daughter nuclei, leaving thus a clear zone in the equator of the spindle. This stained substance was thought to be nucleolar matter dissolved in the nuclear sap. In the subsequent stages these two darker zones move to the equator where the cell plate is built. Its origin Went did not see, but he showed that its growth is peripheral, as had been well established by previous observers. Went, however, observed for the first time that the connecting fibers disappear in the center of the spindle during the growth of the cell plate, so that the peripheral fibers form a ring connected with the growing region of the plate.

Opposed to the view of Strasburger as to the cytoplasmic origin of the cell plate by fusion of swellings of the connecting fibers, was that of Zacharias,<sup>9</sup> who contended that the spindle is of nuclear and the cell plate of cytoplasmic origin, thus following Treub's theory of the origin of the cell plate from free granules. The cell plate is formed by the entrance of substance from the surrounding protoplasm into the equatorial region of the central spindle, which is a portion of the dividing nucleus

<sup>8</sup> Beobachtungen über Kern u. Zelltheilung. Ber. d. deutschen botan. Ges. 247. 1887.

<sup>9</sup> Ueber Kern u. Zelltheilung. Botanische Zeitung 46 : 56. 1888.

(*Mutterkernrest*) having a homogeneous structure and hyaline appearance. In a later contribution he ascribed the filar structure of the central spindle to the effect of reagents, especially absolute alcohol.

In connection with his researches on fertilization Guignard<sup>10</sup> observed that the number of connecting fibers is equal to the number of chromosomes, and that these fibers are formed by the fusion of smaller primary fibers.

Later Strasburger<sup>11</sup> confirmed the views of Went as to the staining properties of the nuclear sap and the relation of the substance in it to cell plate formation. He also described the formation of a temporary plasma membrane about the connecting spindle, and suggested that the change in the form of the nuclear figure, *i. e.*, the bulging out of the connecting fibers, is due to the osmotic action of the nuclear sap held within this membrane.

In 1893 Wildeman<sup>12</sup> described in the rhizoids of mosses a process of division wall formation in which the spindle is first arranged so that its long axis is parallel to the long axis of the cell. Previous to the formation of the cell plate, it is changed to an oblique direction, but the young cell wall is so curved as to become attached perpendicularly to the mother cell wall in accordance with Sachs's law of "orthogonal trajectories."

In 1895 Strasburger<sup>13</sup> claimed new evidence for the identity in character of connecting fibers and other spindle fibers in the fact that their staining qualities were the same. In preparations stained with the triple stain of Flemming he observed that, while the cell plate is being formed, the connecting fibers go through all gradations from violet (the color of spindle fibers) to brown (the cytoplasmic color), thus confirming his earlier opinion as to the cytoplasmic nature of spindle fibers. From the fact that

<sup>10</sup> Nouvelles études sur la fécondation. *Ann. des Sci. Nat. Bot.* VII. 14 : 163.

<sup>11</sup> Histologische Beiträge 1 : 162 *et seq.*

<sup>12</sup> Études sur l'attache des cloisons cellulaires. *Mémoires couronnés et mémoires d. sav. étr. p. p. l'Acad. Roy. d. Sci. de Belgique* 53 : 19. 1893.

<sup>13</sup> Karyokinetische Probleme. *Jahrb. f. wiss. Bot.* 28 : 193. 1895.

shortly before the cell plate is formed "extra-nuclear nucleoli" appear in large numbers in the equatorial region of the spindle, and that by the time the cell plate is complete these have disappeared, he argues that the nucleolus takes part in building the cell plate.

In the pollen mother cells of *Lilium Martagon*, Farmer<sup>14</sup> found that the protoplasm of the connecting spindle on each side of the newly formed cell plate is relatively transparent.

The series of contributions which appeared from the Bonn laboratory in 1897 presented several important facts in regard to the formation of the cell plate. Mottier<sup>15</sup> found in the pollen mother cells of the lily that the cell plate is split into plasma membranes before the cell wall is laid down. In cells plasmolyzed in fixing, a complete plasma membrane was seen around each daughter cell, while no cellulose wall could be observed (*op. cit.*, p. 192). In the pollen mother cells of *Hemerocallis fulva*, Juel<sup>16</sup> found that after the first nuclear division the cell plate did not reach the mother wall but remained free in the cytoplasm until the second nuclear division occurred and new cell plates were formed at right angles to it. Then some of the fibers radiating from the daughter nuclei become attached to the old plate, and all the cell plates now continue their respective growths toward the mother cell wall. Debski<sup>17</sup> studied the division of the segment cells of *Chara* and observed that the long axis of the spindle is parallel to the shorter axis of the cell and further that the cell plate is not formed across the equator of the spindle but nearer one nucleus. In the formation of the ascospores in the asci of *Erysiphe* and *Peziza*, Harper<sup>18</sup> found

<sup>14</sup> Kerntheilung in *Lilium* Antheren, besonders in Bezug auf die Centrosomenfrage. *Flora* 83: 167. 1895.

<sup>15</sup> Beiträge zur Kenntniss der Kerntheilung in den Pollenmutterzellen einiger Dikotylen u. Monokotylen. *Jahrb. f. wiss. Bot.* 30: 169. 1897.

<sup>16</sup> Die Kerntheilungen in den Pollenmutterzellen von *Hemerocallis fulva*, und die bei denselben auftretenden Unregelmässigkeiten. *Jahrb. f. wiss. Bot.* 30: 205. 1897.

<sup>17</sup> Beobachtungen über Kerntheilung bei *Chara fragilis*. *Jahrb. f. wiss. Bot.* 30: 227. 1897.

<sup>18</sup> Kerntheilung und freie Zellbildung im Ascus. *Jahrb. f. wiss. Bot.* 30: 249. 1897.

that the plasma membrane around each ascospore was formed by the growth and lateral fusion of the polar radiations, a process which is essentially similar to the formation of the cell plate in higher plants.

In the division by which the "beaks" are cut off from the gametes in *Basidiobolus*, a typical cell plate is formed, according to Fairchild,<sup>19</sup> from two rows of granules, whose origin was not discovered, in the equatorial region of the spindle. In some of the *Sphacelariaceæ* Swingle<sup>20</sup> confirmed the previous observations of Strasburger, that the cell plate is not formed in a connecting spindle, but arises in the cytoplasmic network. Swingle suggested, however, that its formation may be under the control of the nuclei by means of the kinoplasmic fibers which radiate from the poles, although there are not enough of such fibers actually to form it. An important point in this connection is the fact that if the daughter nuclei are of unequal size the cell plate is formed nearer the smaller nucleus, as Debski described for *Chara*. In the oogonium of *Fucus*, Strasburger<sup>21</sup> found a cell plate built of granules which he considers extra nuclear nucleoli arranged in the cytoplasm. The granules divide so as to form two layers out of which are formed continuous membranes. Discussing the subject more generally, Strasburger<sup>22</sup> places great emphasis upon the kinoplasmic nature of the cell plate. He points out the fact that the formation of a cell plate by the fusion of swellings of the connecting fibers is analogous to the formation of the plasma membrane around the ascospores as described by Harper. In the case of the ascospores there is no doubt of a transformation of the substance of the fibers (polar radiations) into a plasma membrane. In the case of the cell plate, while it is equally evident that there is a transformation of

<sup>19</sup> Ueber Kernteilung und Befruchtung bei *Basidiobolus ranarum* Eidam. Jahrb. f. wiss. Bot. 30: 285. 1897.

<sup>20</sup> Zur Kenntniss der Kern- und Zelltheilung bei den *Sphacelariaceen*. Jahrb. f. wiss. Bot. 30: 297. 1897.

<sup>21</sup> Kernteilung und Befruchtung bei *Fucus*. Jahrb. f. wiss. Bot. 30: 351. 1897.

<sup>22</sup> Ueber Cytoplasmastructuren im Kern- u. Zelltheilung. Jahrb. f. wiss. Bot. 30: 375. 1897.



fiber substance into a lamella from which is derived the plasma membrane of the daughter cells, Strasburger was not then certain whether the cell plate forms simply the plasma membranes, or whether a part of it may be changed into a division wall (*op. cit.*, p. 38). In a subsequent contribution,<sup>23</sup> however, he made the statement that the cell plate forms a plasma membrane (*Hautschicht*), that it splits to form a plasma membrane for each daughter cell, and that the substance of the cellulose wall is excreted by these daughter plasma membranes and laid down as a wall between them.

Hof<sup>24</sup> has recently described in the division of vegetative cells what I take to be the same appearance as that described by Went and Strasburger, viz., the deeply stained portions of the spindle moving toward the equator and there taking part in the formation of the cell plate. He, however, describes the substance of these zones as granular rather than as material in solution in the nuclear sap. After the cell plate is complete the connecting fibers are entirely drawn in (*eingezogen*) and their place taken by alveolar cytoplasm. Just what Hof means by the drawing in of the connecting fibers is a question left unsettled in his description. They may be drawn into the nucleus or into the cell plate. His figures do not help at all in understanding his meaning in this instance.

Nemec<sup>25</sup> has recently described the same phenomena as to the formation of the cell plate in the growing root tips of *Allium Cepa*. He adds further that new connecting fibers make their appearance after these zones have reached the equator. The fact that these new fibers often seem to end free between the original fibers, Nemec considers as evidence for their origin from the stained substance now at the equator.

The observations of Strasburger as to the method of cell plate formation in the oogonium of *Fucus* were not wholly

<sup>23</sup> Zellhäute. Jahrb. f. wiss. Bot. 31: 514. 1898.

<sup>24</sup> Histologische Studien am Vegetationspunkten. Bot. Centralb. 76: 221. 1898.

<sup>25</sup> Ueber die karyokinetische Kerntheilung in der Wurzelspitze von *Allium Cepa*. Jahrb. f. wiss. Bot. 32: 313. 1899.

confirmed by Farmer and Williams.<sup>26</sup> These investigators found that there is an accumulation of material, which they think is some form of a carbohydrate, in the neutral zone between the daughter nuclei of the future oospheres. There is, however, no trace of a preliminary cell plate. While spindle fibers were seen reaching from the poles to the equatorial region of the cell, no evidence was found that they take part formatively in the building of the division wall.

In an early paper Strasburger had pointed out the difference between the method of division of the cell body of animal cells and that of the higher plants, by stating that in the former the connecting spindle fibers take no part in the division of the cell body, but that the division is accomplished by the constriction of the plasma membrane; whereas in the latter these fibers are increased in number and take part in the division of the cell by forming a cell plate. This view Carnoy<sup>27</sup> attempted to overthrow in his work on the testicular cells of some arthropods, in which he claimed to have found a cell plate formed of two parts, a cytoplasmic part formed by thickenings appearing on the junctures of the cytoplasmic network, and a spindle part formed by swellings on the connecting spindle fibers. I have not been able to find that this work has been confirmed by subsequent investigators. The spindle portion (*plaque fusoriale*) of Carnoy is undoubtedly the midbody (*Zwischenkörper*) subsequently described by Flemming<sup>28</sup> and others as arising from granules in the equatorial region of the spindle, but not always as swellings of the spindle fibers. Notwithstanding the fact that it takes no part in the division of the cell body, Flemming was inclined to consider it the homologue of the cell plate in the higher plants. The midbody has received a great deal of attention from the zoologists, and various views have been held as to its significance and fate. The literature on the subject has been

<sup>26</sup> Contributions to our knowledge of the Fucaceæ. Philos. Trans. of the Roy. Soc. London B. 190: 623.

<sup>27</sup> La cytodièrese chez les arthropodes. La Cellule 1: 375.

<sup>28</sup> Neue Beiträge zur Kenntniss der Zelle. Arch. für mikr. Anat. 38: 690.

recently succinctly reviewed by Ballowitz,<sup>29</sup> so that I need not go over it here. Ballowitz finds that the midbody is formed by the fusion of swellings of the central spindle fibers. His observation differs from Flemming's in that the latter thought the granules were sometimes between the fibers. Ballowitz and Flemming agree that the midbody takes no part in the division of the cell, but may be seen between the two daughter cells after division is complete. In a very interesting paper by Kostanecki,<sup>30</sup> upon the relation of polar radiations to the division of the cell body, the view is developed that the division is effected by a cell plate built in the cytoplasm by means of two systems of polar radiations which connect the granular cell boundary with the centrosomes. During the metaphases the longer fibers from each centrosome, which cross each other in the equatorial region of the cell, contract and so change their points of attachments to the plasma membrane until they reach the equator. They now draw in toward the central spindle, pulling in with them a substance similar to that of the cell boundary, thus forming what the author calls an equatorial granular cell plate. The division of the cell depends primarily upon the differentiation of the cell plate into two layers under the influence of the opposite system of polar radiations. Kostanecki states further that he considers the cell boundary to be a material similar to that of the fibers. This hypothesis agrees very well with what we know of the formation of the plasma membrane in the higher plants and asci, as will be seen later. It is perhaps the first suggestion on the part of investigators of the animal cell that the two substances are the same.

### Descriptive.

The present investigation was undertaken to attempt to determine in detail the exact sequence of events during the division of the cell body, and to correlate, so far as possible, the facts

<sup>29</sup> Zur Entstehung des Zwischenkörpers. *Anat. Anz.* 14: 390.

<sup>30</sup> Ueber die Bedeutung der Polstrahlung während der Mitose u. ihr. Verhältniss zur Theilung der Zelleibes. *Arch. f. mikr. Anat.* 49: 651.

thus brought out from the point of view of the physiology of cell reproduction.

#### MATERIAL.

My observations were made largely upon (1) vegetative cells found in the growing root tips of various phanerogamic plants, among which may be mentioned *Allium Cepa*, *Lilium longiflorum*, *Fritillaria imperialis*, *Hyacinthus orientalis*, *Vicia Faba*, *Phaseolus vulgaris*, *Pisum sativum*; and (2) pollen mother cells of *Larix Americana* and *Larix Europæa*, and dividing pollen grains of *Iris versicolor* and *Hemerocallis fulva*.

In the first group I found the liliaceous plants were much more favorable for observation, and of these, owing to the ease with which the material could be obtained, *Allium Cepa* was the one generally used. I have satisfied myself, however, that the phenomena I shall describe occur in all of the plants mentioned, and my conclusions are of course drawn from a study of all of these plants. In many particulars there is a notable difference between the phenomena that may be seen in such tissues as the root tip and such cells as the pollen mother cells of the larch. These differences I shall attempt to correlate to some extent with the nature of the cells.

#### METHODS.

As some of the phenomena herein recorded are at variance with those observed by previous investigators, I have thought it worth while to give a detailed account of the methods used, even though they are those already well known to cytologists. Various killing and fixing methods were employed. Flemming's chrom-osmo-acetic acid (both formulas); Hermann's platinum chlorid-chrom-acetic acid; Vom Rath's platinum chlorid-picro-osmo-acetic acid; Keiser's mercuric chlorid-acetic acid; 96 per cent. alcohol, and a mixture of mercuric chlorid, formalin, acetic and formic acids arranged by Professor D. C. Worcester, of the department of zoology of the University of Michigan, and soon to be published by him. Of these methods, the one best adapted to my purpose was the stronger solution of Flemming,

since the kinoplasmic portions of the cell in division were nearly always well fixed in it. It proved in this respect to be much superior to the weaker solution for the tissues studied. The material was kept in the killing fluid 30-48 hours, washed in running water, hardened by carrying through the different grades of alcohol and imbedded in paraffin in the usual way. Some care was necessary to avoid plasmolysis in the early stages of hardening. I found that the objects could not be left in the lower grades of alcohol for a very long time; 30 minutes in 15 per cent., 45 minutes in 30 per cent., and one hour in 50 per cent. seemed to be about the maximum limits. Above 50 per cent. more latitude as to time could be allowed.

Vom Rath's and Hermann's solutions generally fixed the material well, but were not so useful as Flemming's solution on account of the blackening of the tissue and the consequent difficulty in getting a clear differentiation in staining. The method of Professor Worcester was found to be useful for comparative purposes. For these tissues it has no other special value. Alcohol (95 per cent.) was irregular in its effects, sometimes giving well-preserved material but more frequently causing plasmolysis. The other methods were found generally unsatisfactory.

The sections (generally  $5\mu$  thick) were cut on a microtome and stained on the slides. The method best adapted to use in connection with objects killed in fluids containing osmic acid was the safranin-gentian-violet-orange method of Flemming. After removal of the paraffin the slides were placed in the safranin solution 25-30 minutes, rinsed in water, and then put into the gentian-violet solution 20-25 minutes. They were again rinsed in water and passed rapidly through a *weak* orange solution (1 part saturated solution orange G in  $H_2O$  + 1 part  $H_2O$ ), dehydrated, clarified quickly in clove oil, and mounted in balsam. For staining objects killed in the fluids containing mercuric chlorid, Zimmermann's fuchsin iodine green<sup>31</sup> and Heidenhain's haematoxylin, preceded by Bordeaux red as a ground stain, were found most serviceable.

<sup>31</sup> Morph. u. Phys. d. pfl. Zellkernes 6.

In order to show clearly certain zonal differentiations in the spindle as well as other general structures in the cells I have used photo-micrographs for many of the illustrations. I have considered this the more satisfactory, inasmuch as my preparations have showed some phenomena varying markedly from the descriptions of other observers.

#### OBSERVATIONS.

For purposes of description we may group the phenomena under three heads representing three stages in the development of the cell plate, viz., *preparatory*; *genetic*; *growing*.

##### 1. *The preparatory stage.*

At the time when the chromosomes have collected at the equator to form the equatorial plate the spindle is of the form that Hof has recently termed "monaxial," *i. e.*, a spindle having a single axis but not necessarily ending in single definite points as poles (*figs. 1, 10, 23*). The poles of the spindle in both the larch and the onion are in most cases somewhat blunt. In the latter plant my observations agree entirely in this respect with those of Hof (*l. c.*) and Nemec (*l. c.*). In the case of the larch I have not confirmed Strasburger's<sup>32</sup> observation of a centrosphere. In my preparations the poles generally appear blunt as they do in the onion.

The fibers which appear in connection with this stage may be grouped into three systems, connecting fibers, mantle fibers, and radiating fibers. These systems correspond in general to the system of fibers similarly named by older investigators, especially among the zoologists, except that here, as will appear later, the distinction cannot be so sharply drawn between connecting fibers and radiating fibers. If we study the above mentioned systems of fibers more in detail, it will appear that while many of the connecting fibers are collected into thick strands or bundles, and others appear as single fibers, such an arrangement does not indicate a necessary difference of character. Very frequently a single fiber diverges from a bundle and

<sup>32</sup>Karyokinetische Probleme *fig. 25*.

unites with another bundle or continues as a single fiber to the pole. The arrangement into bundles seems to be produced partly, at least, by the fibers being crowded into the small spaces between the chromosomes. This explanation is rendered more probable by the fact that, as Strasburger has pointed out, the arrangement into bundles is much more evident at the equator than in the polar regions of the spindle (*op. cit.*, p. 183), *i. e.*, the fibers forming a single strand often seem to diverge beyond the equator. Some of my preparations show that this divergence reaches to the ends of the strand (*fig. 23*), so that such strands are most compact where they are adjacent to the chromosomes.

The appearance of single fibers crossing obliquely from one bundle to another, together with the fact that such fibers often cross and recross each other, was taken by Belajeff as evidence that the fibers are drawn out portions of a protoplasmic network. Guignard's observation that the number of connecting fibers is equal to the number of chromosomes may be explained by the hypothesis that the fibers are collected into larger strands in the spaces between the chromosomes. Guignard, as previously stated, described such fibers as secondary fibers formed by the lateral fusion of previously existing smaller primary fibers. He did not, however, distinguish any other than the connecting fibers. Strasburger later showed that there are other fibers extending from the poles to the chromosomes. These are the mantle fibers. They have been so clearly demonstrated recently by Osterhout, Mottier, Nemec, and others, that I need give no detailed description of them in this connection. Suffice to say, I find no apparent difference in structure between them and the connecting fibers.

The third system of fibers to which I have referred may be described briefly as including those which extend from the poles into the cytoplasm. They are much more abundant in the larch than in the onion; hence I shall first describe them as they appear in the former. They seem to have the same structure as the connecting or mantle fibers. Strasburger has figured them in the larch as radially arranged granules, while the connecting

fibers are shown as continuous lines. In my preparations the granular trophoplasm through which the fibers extend often has a radial appearance where the fibers are very abundant, but it is always possible in good preparations to distinguish the single threads (*fig. 23*). It is only by means of their distribution and arrangement that I would distinguish the radiating fibers from the other systems. They may be said to be centered in a general way on each pole, and to radiate through the cytoplasm in all directions toward the cell boundary, but at this stage very few appear to reach the plasma membrane. Their arrangement around the poles is by no means regular. Some of them lie across the poles in such a way as to have both ends free in the cytoplasm. With these it is of course often difficult to determine whether a single fiber is seen, or whether the appearance is given by two fibers extending from the same point in opposite directions into the cytoplasm. In many cases, however, I have been able to satisfy myself that a single fiber extended through the pole and that its two ends lay free in the cytoplasm (*fig. 23*). This was most evident in cases where the fibers lay in such positions as to form appreciable angles with the axis of the spindle. Where the apparent radiating fibers are continuous or nearly so with the long axis of the spindle it is often difficult to determine whether they extend through the poles into the region of the spindle itself or whether they end at the poles. In some cases it appears as if such fibers are merely prolongations of the connecting or mantle fibers. I could not be certain on this point on account of the abundance of fibers in the polar regions of the spindle. Some, however, may be traced through the poles into the cytoplasm closely adjacent to the spindle. Still it can be readily observed that many of the radiating fibers which lie closely adjacent to the spindle do not extend beyond the poles. These latter fibers bear an interesting relation to the connecting fibers. They often extend from the pole parallel to the connecting fibers, even uniting with the strands of the latter, nearly to the equator, where they curve outward and end blindly in the cytoplasm. Many of the fibers from each pole cross one



another at the equator. These generally appear to be longer and more abundant than any of the other radiating fibers, but their identity in appearance with the connecting fibers makes it impossible in many cases to determine whether they extend to the poles or not.

From the above observations it seems to me to be improbable that there is any fundamental difference between the fibers of the three systems. While for convenience of discussion it is perhaps best to retain the classification heretofore used, it is worth while to understand clearly just what significance is to be attached to the different terms. By connecting fibers I mean those which lie entirely in the spindle and extend *across the equator*. Mantle fibers are those which lie entirely within the spindle and are attached to the chromosomes. The spindle is understood, then, to be made up of the mantle fibers and the connecting fibers. Radiating fibers are those which, connected in small part with the spindle, generally if not always at the poles have at least one end lying free in the cytoplasm. That this definition of radiating fibers may have to be slightly extended will appear later.

Whether there is any difference in origin of the various fibers I have not attempted to discover. The recent researches of Belajeff,<sup>33</sup> Osterhout, Mottier, and others, have indicated that all of the fibers have a common method of origin. Rosen,<sup>34</sup> however, believed from his study of spindle formation in the root tips of *Hyacinthus orientalis* that the connecting fibers are formed each by two fibers growing from opposite directions and fusing end to end at the equator. His observations have not been confirmed by later investigators.<sup>35</sup> So far as my observations go, in the onion radiating fibers seldom appear at this stage. Nemec, however, has described them as first appearing in the very early prophases and persisting through the metaphases. Their history, as Nemec describes it, is of much interest. The first

<sup>33</sup> Zur Kenntniss der Karyokinese bei den Pflanzen. *Flora* 79: 436. 1894.

<sup>34</sup> Kerne u. Kernkörperchen in meristematischen u. sporogenen Geweben. Cohn's Beiträge zur Biologie der Pflanzen 7: 225.

<sup>35</sup> HOF, *loc. cit.*, and NEMEC, *loc. cit.*

indication of a spindle is an accumulation of hyaline substance around the nucleus, but in greater amount on the sides corresponding to the poles of the future spindle. The whole body thus forms an ellipsoid spindle fundament (*Anlage*). This soon shows a filar structure and in many cases these fibers extend outward from the sides and cross in the equatorial region of the cell. The radiating fibers thus appear prior to the other fibers, or it would perhaps be more accurate to say that they grow more rapidly, as rudiments of the other fibers appear at the same stage in the polar regions. It should be noted here that no radiating fibers are described for these early stages, except those extending toward the equator. Such fibers, however, reach to the plasma membrane and are attached to it. The attachment is shown by a small accumulation of violet-staining material at the point of juncture. While Nemec states that the above mentioned fibers persist throughout the process of nuclear division to the beginning of the anaphases, his figures of the equatorial plate stage do not show them, but in connection with the late metaphases they are again figured. It seems to me probable that the two sets of fibers are not identical. It is not unlikely that those first described changed their position and became connecting or mantle fibers, while the later radiating fibers are a new growth. Nemec himself states that he believes that some new radiating fibers are formed during the late metaphases. I have been able to see some radiating fibers in the onion in connection with the equatorial plate stage, but not in abundance nor of such length as Nemec described. They seldom reached beyond the equator. When they were present they showed the same relation to the other spindle fibers as has been described for the larch.

The appearance of the cytoplasm in this stage requires a brief description. In the onion, it surrounds the spindle closely and may often be seen between the fibers in the polar region. It is generally slightly denser in the vicinity of the spindle than it is nearer the cell-membrane. In older cells there are frequent vacuoles in the outer portion. In the larch an interesting differentiation frequently occurs. About midway between the cell

periphery and the spindle there is a thin layer of kinoplasmic material, looking as if it were made up of small portions of fibers in a tangled mass (*fig. 23*). A similar appearance was described by Mottier (*op. cit.*, p. 180) in an earlier stage of spindle formation in the pollen mother cell of the lily. He observed, however, that this layer forms a part of the spindle, *i. e.*, it is the same as the felted layer described by Strasburger and Belajeff. That it forms no part of the existing spindle in the larch, however, would be indicated by its late appearance after the spindle is formed. It may be that there is in the larch more kinoplasm formed than is needed for this stage in the development of the spindle, and that it becomes absorbed into the cell protoplasm or is used directly for the later stages in the growth of the fibers (p. 47.) Between this kinoplasmic layer and the spindle there often appears a thick layer of finely granular trophoplasm which stains readily with the orange. A similar layer in the pollen mother cells of *Hermerocallis* was described by Juel, but he did not mention any strong affinity for the orange stain, such as I have observed in the larch. In some of my own preparations of *Hermerocallis*, I have observed the orange-stained layer very frequently.

Throughout the cytoplasm, and in some cases in the spindles also, there often appeared, in this as well as in later stages, large blue-stained granules. Their distribution was irregular. They were more apparent usually in the larch than in the onion, except during the metaphases, when they appeared very abundantly in the spindle of the latter. I shall discuss their appearance in connection with such stages in a subsequent paragraph.

In the early metaphases the connecting fibers appear of course much more plainly between the receding groups of daughter chromosomes. They can easily be traced through to each pole, thus being identified as the connecting fibers before mentioned and not as secondary structures formed simply between the groups of chromosomes. They show in the larch the compound structure before mentioned. In the onion, on the other hand, the portions between the receding groups of daughter

chromosomes often appear as single coarse fibers. This appearance is probably due to the closer crowding of the small fibers by the chromosomes. It is possible that in some cases this crowding together has gone to such an extent as to result in an actual lateral fusion of the fibers, such as Guignard suggested. The above-described condition of connecting fibers is doubtless what led Nemec to conclude that during the metaphases there were new coarse fibers formed reaching merely between the daughter chromosomes. I have been able to determine that in most cases the fibers can be traced through to the poles, and that they often appear to separate into smaller fibers beyond the chromosomes in the way described by Strasburger for the larch. In the larch, in which the fibers show their compound character much more clearly, the true relation is still more distinctly seen (*fig. 2*). While I have not been able to confirm the observations of Nemec (*op. cit.*, p. 329) as to the presence of secondary fibers during the metaphases which are formed merely between the groups of daughter chromosomes, I have often observed that the above-mentioned blue-stained granules appear to be very numerous in the region of the spindle. They often appear in rows and sometimes seem to be sticking to the connecting fibers. But such a regular arrangement is not at all characteristic. They are as frequently scattered in the ground substance of the protoplasm (*fig. 25*). That they are formed from disintegrating secondary fibers seems impossible to believe when we consider the fact that they may be scattered throughout the whole cell as described above. As to the function or ultimate fate of these granules I could learn nothing definite. They seem to be distinct from the ordinary granules of the trophoplasm in staining qualities alone. The fact that they showed most plainly in the region of the spindle may simply mean that they are more readily distinguished by form from the spindle fibers, which in this stage are not abundant, than they are from the other granules appearing in the cell.

While the above mentioned processes have been taking place in the connecting fibers, the radiating fibers and the cytoplasm

have undergone important changes. In the larch the radiating fibers apparently grow longer, so that by the time the chromosomes have reached the poles a great many of them may be traced to the plasma membrane. Any evidence of such a fusion of the fibers and the plasma membrane as Nemec described in the onion I was unable to find. In the equatorial plate stage the fibers certainly did not reach to the plasma membrane. Whether the difference in appearance of the radiating fibers is really due to the growth of the existing fibers or to the formation of new ones, I could not determine. In the later stages, shorter fibers may be seen, which are possibly the radiating fibers of the earlier stages, while the longer fibers are newly formed. The increase in number of fibers which such an explanation would demand was, however, not always evident in my preparations. In connection with changes in the radiating fibers there is a disappearance of the kinoplasmic layer heretofore described as lying between the spindle and the cell periphery. It may be, as previously suggested, that the substance is used in the growth of the radiating fibers, though I have no positive evidence that such a relation exists. The fine granular zone also disappears during the metaphases. There is now a tendency for the cytoplasm around the poles of the spindle to assume a finely granular appearance. The significance of such an appearance I have not attempted to explain.

In the onion the relation of the trophoplasm to the spindle remains practically the same as it was in the equatorial plate stage. When the diaster is formed there is a small number of connecting fibers, and the few radiating fibers that existed during the previous stage have not appreciably changed in appearance. During the later stages of the diaster, however, there is an apparent growth of new radiating fibers extending in all directions from the daughter nuclei. This phenomenon I shall discuss in a subsequent paragraph.

Following the formation of the diaster there sets in a series of activities which are concerned immediately with the formation of the cell plate. In the larch, the spindle soon appears to be

differentiated into three zones (*fig. 2*), as Went first pointed out in other forms. But I cannot confirm the statement that the darker subnuclear zones take their characteristic appearance from a stainable ground substance between the spindle fibers. A careful study of the preparation from which *fig. 2* was taken has convinced me that the darker portions are due to the structure and arrangement of the spindle fibers themselves. Some of the fibers are undoubtedly thicker in this region than in the equator (*fig. 27, a*). With this differentiation in structure is combined the fact that the bundles have here begun to separate into single fibers (*fig. 2*). The two processes taken together account for the extra density observed. The above facts seem to me to indicate that the kinoplasmic activity preparatory to the formation of the cell plate begins in the region of the nuclei. The thickened appearance of the fibers soon extends throughout their length (*figs. 3* and *27, b*). Concurrent with such thickening the separation of the bundles into single fibers continues until the central spindle has the same appearance throughout. By comparing *figs. 2* and *3* it can be seen that nearly all of the connecting fibers have apparently shortened slightly, leaving clear spaces just under the two daughter nuclei. In many cells there were often observed single connecting fibers in which the above described changes did not seem to be taking place, but they were never numerous in any one cell. Their distribution was not regular. They were as often seen in the central as in the more peripheral parts of the spindle. I shall have occasion to refer to them in the description of later stages. Granules of trophoplasm may often be detected among the ends of the connecting fibers at this stage. These have probably flowed in as the fibers contracted. The process of separation of the fibers described above gives the appearance of an increase in the number of spindle fibers. Whether such an increase actually occurs is doubtful. In the larch the evidence, so far as I have observed it, seems to show that the apparent increase is due entirely to the above mentioned processes. In this connection the hypothesis lately suggested by Strasburger, that the spindle fibers are increased in

numbers by splitting, is of interest. His evidence for the hypothesis may be stated as follows: (1) the apparent rapid increase in the number of fibers; (2) the fibers produce a rapid outgrowth of the cell plate; (3) they are often found lying closely side by side. The first and third points seem to me to be readily explained by the facts that I have already described for the larch, viz., the rearrangement of the fibers by the bundles separating into single fibers, and the shortening and thickening of the fibers, resulting in a spindle of denser appearance, with apparently more numerous fibers. Strasburger himself points out that the spindle fibers seem to separate from the daughter nuclei and to become thicker and more densely stained prior to the formation of a cell plate. It should be noted, however, that Strasburger had previously accepted Guignard's doctrine that the connecting fibers are secondary structures formed by the fusion of smaller primary fibers. The splitting of the fibers, in Strasburger's sense, would be essentially the same as the separation of fibers, as described above, with this one exception: in the former case the process is unlimited, for new fibers may continuously arise by the splitting of original fibers, but in the latter the process is limited by the number of fibers making up the bundles. If the splitting hypothesis were true, it would explain, as Strasburger suggested, the appearance of new peripheral connecting fibers, and thereby the growth of the cell plate; but below I shall describe phenomena which seem to indicate that the appearance of new peripheral fibers depends not upon such a multiplication of the original connecting fibers, but upon changes in some of the radiating fibers.

Concurrent with the above described changes in the connecting fibers, the distal portions of the radiating fibers that lie nearest the central spindle and cross at the equator seem to bend in such a way as to come nearer together and to give the appearance of bowed out connecting fibers; but that they are the previously described radiating fibers is evident from a careful study of the preparations. In *fig. 26* I have drawn accurately two such fibers extending one from each nucleus. Here the

relation is such as to indicate that the two fibers have fused laterally throughout a part of their length. Whether such a fusion is real or only apparent, I could not determine. A careful study of the preparation from which the photograph for *fig. 2* was made shows all stages in the arrangement of radiating fibers, from that shown in *fig. 26* to that in which the fibers extend out into the cytoplasm and cross at the equator in such a way as to form sharp angles (*fig. 24*). The significance of these facts I shall discuss in a later connection. The radiating fibers that do not cross at the equator, so far as I could determine, have suffered no appreciable change. It is important to keep in mind that, with the exception of the presence of the trophoplasmic granules, the changes in the appearance of the spindle which have so far taken place in the larch are due mainly to changes in the existing spindle fibers themselves, and not to the addition of new fibers or of other material.

If we turn now to the onion, we note that there is an apparent increase in the number of connecting fibers (*figs. 12, 13, 14*). *Fig. 13* represents the same stage in the onion as that represented by *fig. 2* in the larch. The slight differentiation of the spindle into zones may be seen. While the smaller number of spindle fibers renders such a differentiation less conspicuous, it is evident that it is caused in the same fashion as in the larch. A fact of importance to note here is that there are now visible more radiating fibers than could be seen in earlier stages. The photograph has not brought these out very clearly, but they may be seen by close inspection. These fibers radiate in all directions from the nuclei, but those are more abundant which extend toward the equator. In this connection it may be worth while to point out that these new fibers do not bear exactly the same relation to the spindle as the radiating fibers that exist in the equatorial plate stage. Those were centered not on the nuclei or chromosomes but upon the poles of the spindle. It is possible, however, that they were originally centered upon the mother nucleus, as previously suggested. From a careful comparison of this with earlier stages, I am convinced that there



has been a growth of new fibers. It is not impossible that some of these extend into the central spindle, thus increasing the appearance of zonal differentiation, but I could not establish this by actual observation. In an earlier paper Guignard figured such a relation but did not describe it. It seems to be not improbable that such a condition may exist, and that such fibers may grow in length and form new connecting fibers, either by fibers from the opposite nuclei fusing, or by a continuous growth from one nucleus to the other. This process would account for the apparent increase in number of connecting fibers. Still the evidence is too meager to lead to any definite conclusion. There is no convincing evidence that there is any real increase in the number of connecting fibers. Its appearance may be due entirely to the changes which take place in the original fibers, as is the case in the larch. On the other hand, the relatively small number of the connecting fibers in earlier stages seems to show that some new ones have been formed. The question needs further investigation. If there are new connecting fibers formed they seem to act simultaneously with the original fibers in the process of forming the cell plate. The point that seems of most importance to me here is the previously described relation of the radiating fibers to the daughter nuclei, *i. e.*, that they center not upon the poles of the spindle but upon the nuclei themselves. This relation, combined with the fact that the fibers are new formations, may indicate that the nuclei are the metabolic centers for the formation of spindle fibers. Such an hypothesis is further strengthened by the previously described changes in the connecting fibers in the larch, where the increased thickness is first evident near the ends of the fibers, *i. e.*, in those portions nearest the nuclei. The fact that the appearance of the new fibers comes prior to the reconstruction of the daughter nuclei does not invalidate the above hypothesis, for, as Juel has shown in the formation of abortive pollen grains in *Hemerocallis fulva*, single chromosomes may have spindles formed between them and the normal nucleus. Here would seem to be a case in which a chromosome as such

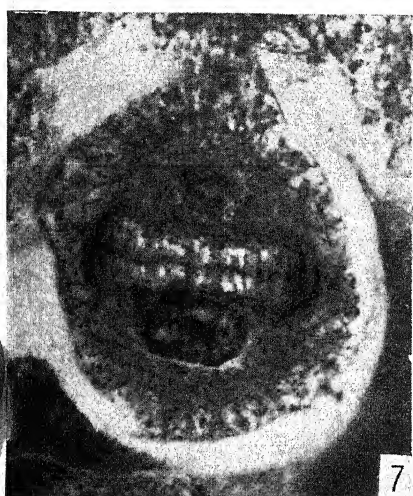
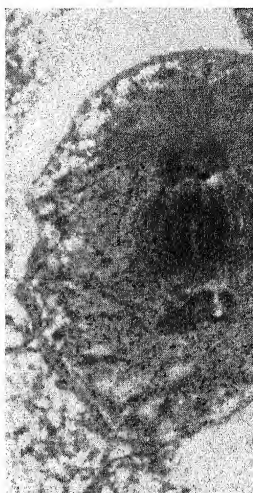
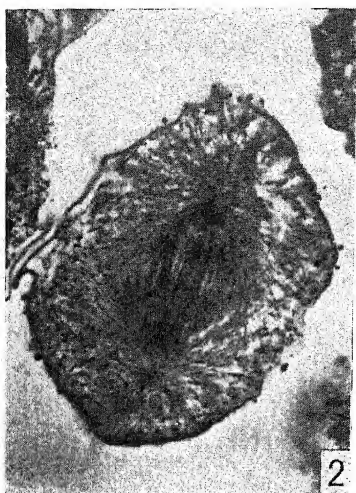
acts as a center for spindle formation. While the recent contributions on the subject of spindle formation in higher plants vary greatly in detail they all agree in one particular, viz., that the first indications of a spindle are to be found in connection with the nucleus and that the subsequent prophases take place while the fibers remain in this connection. These facts may be explained by the hypothesis that the nucleus is the center for the formation and activity of the kinoplasm.

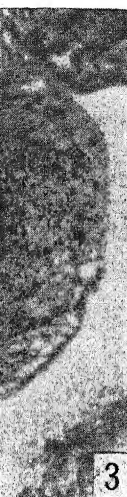
The further history of the equatorial zone in the onion presents a striking difference from its history in the larch. Whereas in the latter it disappears with the rearrangement and change in the connecting fibers prior to the formation of the cell plate, in the former it soon becomes filled with a substance that stains strongly with the orange of the triple stain. The spindle fibers retain their violet color in the same regions, showing that the orange stain is taken by a substance foreign to the fibers (*figs. 14, 15, 28*). In appearance this substance is entirely homogeneous. I could detect no appearance of granules or any other definite structural elements in it. It would seem to be a substance in solution in the living cell. It is a significant fact that the cell walls showed the orange stain in those preparations in which this orange stained substance is most evident in the spindle. In many preparations in which there was a slight excess of violet, the cell walls were stained blue and the substance in the equatorial zone could scarcely be distinguished from the surrounding blue stained protoplasm. By the use of other stains it was often difficult to color or differentiate this substance. In one preparation, stained with eosin and methyl green, in which the methyl green was in excess, all parts of the cell were stained green except the equatorial zone and the cell walls, both of which showed a slight tinge of eosin. In all of the stains used when both the cell wall and the interfilar substance were stained, their color was the same. There were some cases, however, in which the walls were stained while the equatorial zone remained colorless, *e. g.*, slides stained in ruthenium red or iron haematoxylin.

The similarity in staining of this substance to that of the cell walls, together with its presence in the region of the spindle in which the cell wall appears later, I have taken to signify the presence of a carbohydrate substance destined for the formation of the new cell wall. Whether it is at all analogous to the previously described layer around the spindle in the cytoplasm of the larch, I am not able to say. The fact may be of some significance in this connection, that in a late stage of division in the larch a similar orange layer appears in the cytoplasm around each daughter nucleus. Possibly it shows the presence of material destined for the formation of the walls of the pollen grains. This fact may be correlated with the fact that the division of the pollen mother cell often does not follow the first nuclear division. But in the stages showing the young permanent cell plate, such a substance was generally invisible, though in some cells a slight indication of it was seen. There would seem to be in these cells very slight or no aggregation of reserve cell-wall material. It is interesting to note that in the dividing pollen grains of *Iris* and *Hemerocallis* no orange stained inter-filar substance was seen. Here, of course, there is no cell wall formed between the two cells.

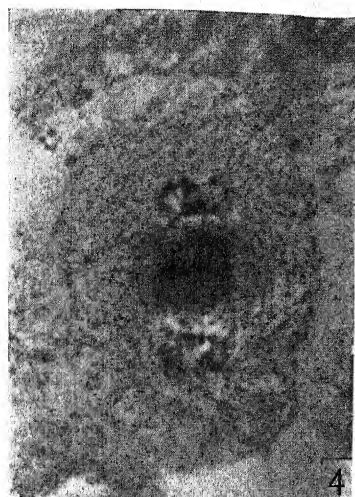
The relation of the spindle fibers to this orange substance is worthy of notice. As above mentioned, they retain their characteristic color, but they often appear greatly attenuated in this region. It would seem that the substance had crowded them into such a condition (*figs. 28, 29*), and that it retarded the previously described processes of thickening and separating of the fibers.

The staining qualities of the inter-filar substance agree with what Farmer and Williams have described for the protoplasm of the neutral zones between the daughter nuclei of the future oospheres of *Fucus*. In the *Saprolegniaceæ* Trow has suggested that the so-called cellulose granules are a form of reserve cellulose, which may be used to close the opening made by the breaking of a hypha. I have seen preparations of *Saprolegnia* in which the young cell wall cutting off the sporangium stains strongly with





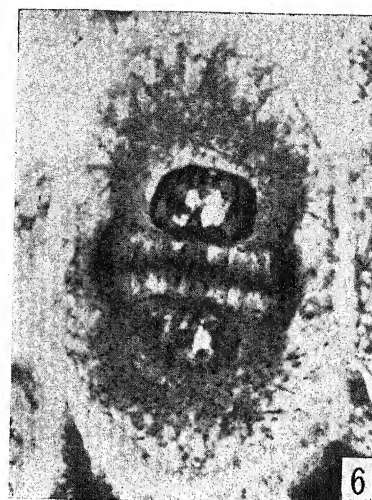
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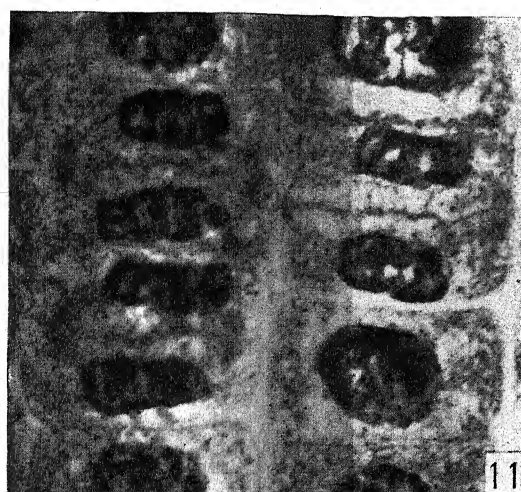
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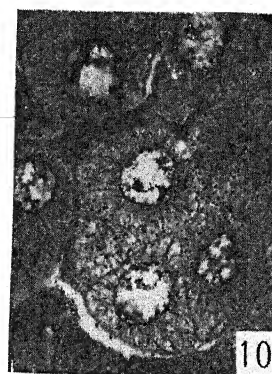
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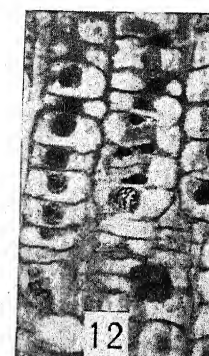
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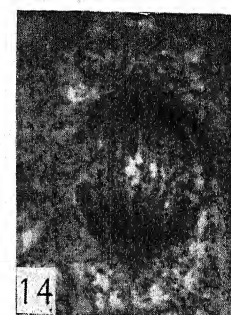
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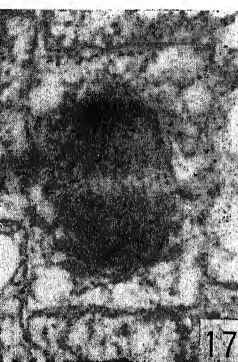
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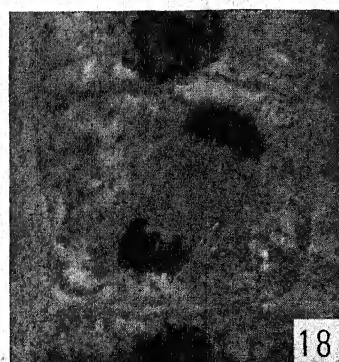
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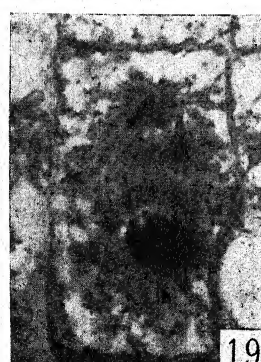
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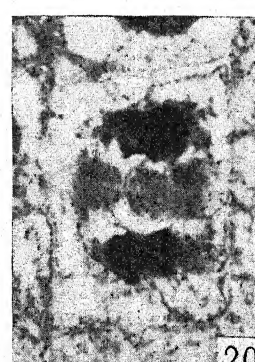
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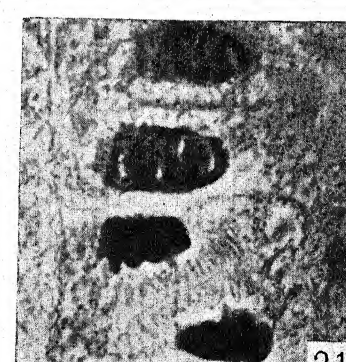
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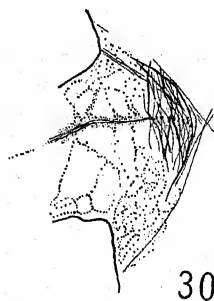
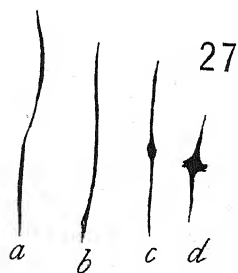
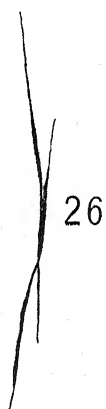
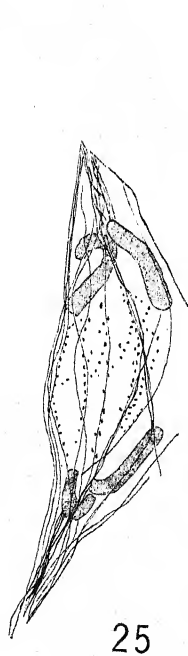
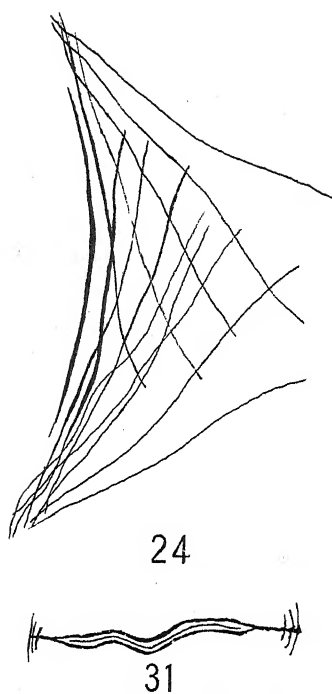
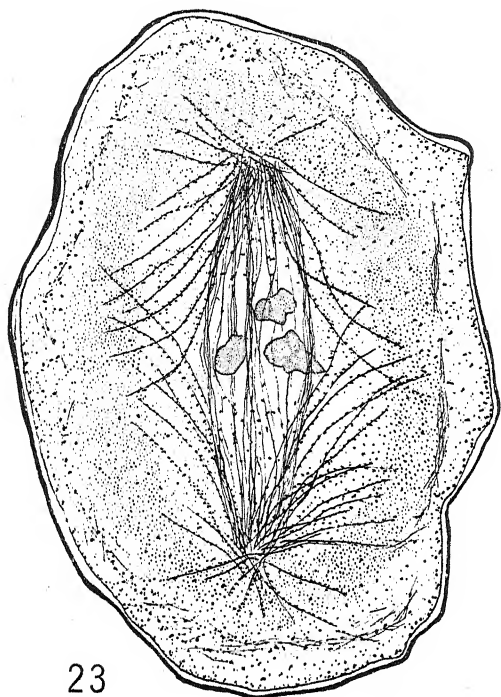
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TIMBERLAKE on CELL PLATE

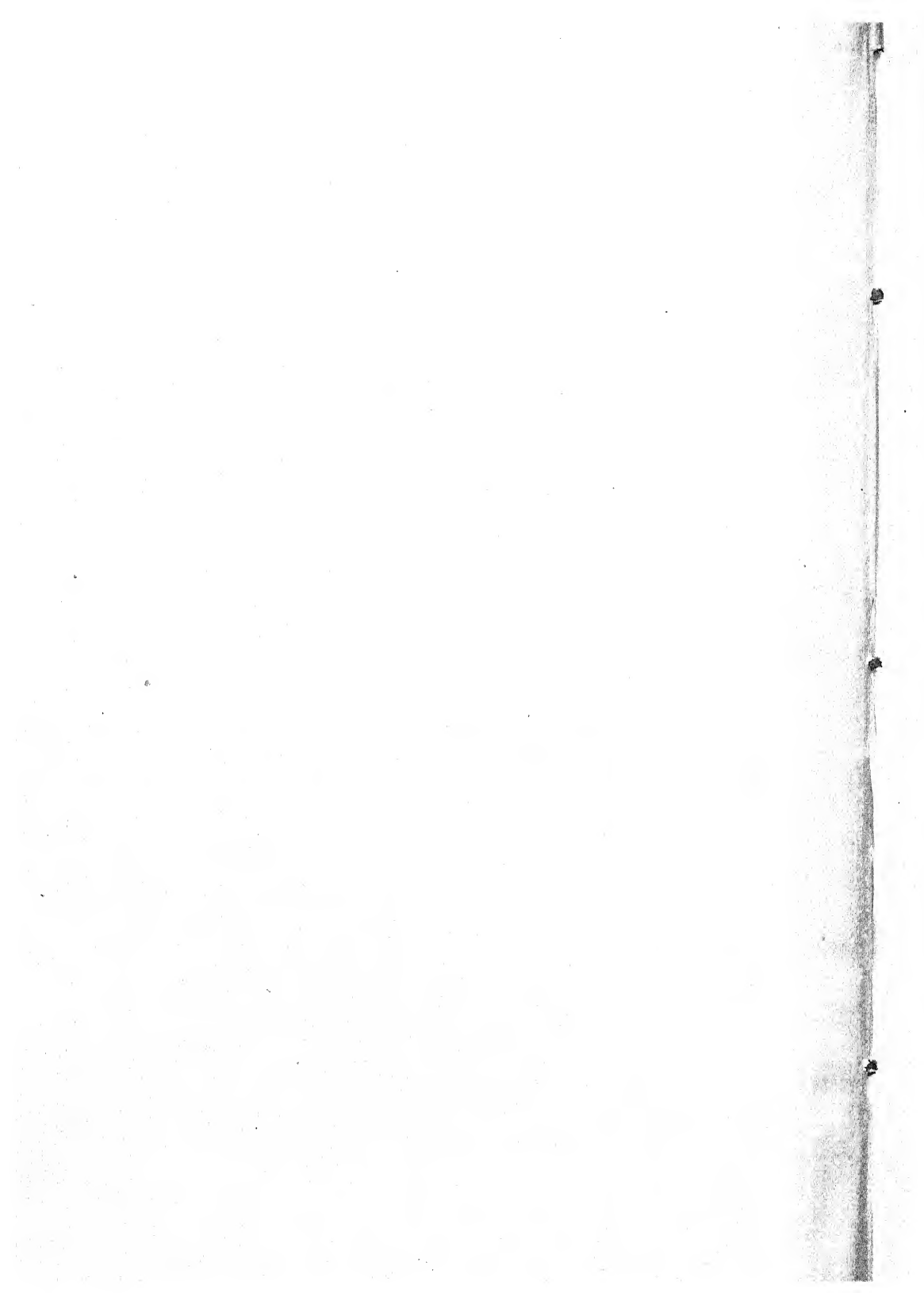


BOTAN





TIMBERLAKE on CELL PLATE





orange, and strongly orange stained granules are often apparently imbedded in the adjacent thick plasma membranes. The above facts seem to indicate that there may be in the protoplasm some form of reserve carbohydrate in readiness for the formation of a cell wall. I shall hereafter use the term carbohydrate material in speaking of the interfilar substance. The further history of this material is connected with the subsequent stages in the development of the cell plate. The essential difference thus far between the larch and the onion is that in the former the processes preparatory for cell division have been mainly carried on in the already existing fibers, while in the latter there has been a formation of new fibers and an aggregation of carbohydrate material in the equatorial region of the central spindle.

UNIVERSITY OF WISCONSIN.

*(To be concluded.)*

CONTRIBUTIONS FROM THE CRYPTOGAMIC LABORATORY OF HARVARD UNIVERSITY. XLIV.

NEW OR LITTLE KNOWN UNICELLULAR ALGÆ.  
I. CHLOROCYSTIS COHNII.

GEORGE THOMAS MOORE.

(WITH PLATE X)

EVER since the discovery by Cohn, in 1872, of the chlorophyllous endophyte, *Chlorochytrium Lemnæ*, there has been considerable interest in algæ having such a habit, and much speculation has been indulged in, both as to their affinities and the method whereby they acquired their peculiar condition.

Probably the first recorded instance of a green alga living within the tissues of a host was that described by Mettenius (13) in 1850. This author found that in the fronds of *Polyides rotundus* (Gmelin) Grev. were curious green cells which were more or less crowded together and completely surrounded by the substance of the alga. These cells Mettenius considered to be the mother cells of the spores of *Polyides*, but Cohn (1) thought it more likely that they were young plantlets of some grass-green alga. Thuret (1) in a letter to Cohn confirmed this view and identified the plant as *Cladophora lanosa* (Roth) Kütz. He explains that he found the germinating zoospores within the cortical tissues of the *Polyides*, which gradually increased in size without dividing. Towards the end of the winter, however, they elongated rapidly, breaking through the tissues of the *Polyides*, and developed into small *Cladophora* tufts. Cohn found similar plants at Heligoland, but did not observe that they became septate or grew through the tissues of the host plant; hence he regarded them as being distinct endophytes. Some years previous to this, Cohn (2) described some long, narrow, green cells which occurred among the densely packed filaments of *Petrocelis cruenta* J. Ag. He at first regarded these as the

normal reproductive cells of the *Petrocelis*, but later came to consider them as something quite separate from this plant. These green cells growing with *Petrocelis* have been found in this country by Dr. Farlow (6), and recently Kuckuck (11) has decided the plant to be a *Codiolum*, having no distant connection with its supposed host.

After *Chlorochytrium* was described on *Lemna* a number of endophytic forms were discovered, some of which showed such marked resemblances to certain fungi that, had it not been for their green color, they would undoubtedly have been placed within that group. One of these "green parasites," as they were popularly termed, was found by Wright (17) in 1876 growing on various algæ off the coast of Ireland, and called by him *Chlorochytrium Cohnii*. The discoverer of this form was so impressed with its fungus-like appearance and habit that he devoted considerable space to the discussion of how the plant was in reality a fungus which had but recently acquired the property of manufacturing chlorophyll. He was even able to observe the stages in this process as the plant developed. It is not my intention to go into a discussion of how fungi and endophytic algæ are related to each other; I merely wish to describe one of the algal forms, and any comparisons to be made with the fungi must be left to another time.

While collecting along the beach at Lynn, Mass., in February 1897, my attention was attracted to the peculiar granular appearance of some *Enteromorpha* which was growing attached to piles. When brought into the laboratory and examined under the microscope, the alga was seen to be covered with a green unicellular organism which at first did not seem to have been previously described. Upon more careful examination and an exhaustive search of the literature upon the subject, it was thought that this plant must be the endophytic alga found by Wright, *Chlorochytrium Cohnii*. The material collected by me, however, did not perfectly agree with any published account of this species, and according to the keys in both De Toni (3) and Engler and Prantl (5) could not find a place within that genus.

It seemed necessary, therefore, to study the structure and development of the plant more carefully before it could be decided whether or not it really was a new genus.

Since the plant was discovered by Wright, there have been but three published accounts of this form, for, although it occurs in widely separated regions and upon a number of different hosts, it is but rarely collected. Lagerheim (12), in 1884 found it off the coast of Sweden, and the next year Reinhardt (14) came upon it near Sebastopol, while investigating the flora of the Black Sea. The latter observer considered that the variations in this form from that of the original *Chlorochytrium* described by Cohn were sufficient to place it within a new genus, and he consequently proposed the name *Chlorocystis* which it has since borne. In 1892 de Wildeman (4) secured material from off the coast of France and published a short account of the plant. It has been reported from Greenland by Rosenvinge (15) and a new species growing on *Sarcophycus potatorum* has been described by Miss Whitting (16).

The material collected at Lynn frequently showed the *Enteromorpha* to be so nearly covered by the *Chlorocystis* as to cause it to appear rough and somewhat distorted even with a hand lens (figs. 1 and 2). The smaller more delicate pieces of the host plant seemed to be a more favorable resting place for the unicellular alga, and it was only occasionally that an individual was found upon the larger more exposed plants. The only reason discernible for this was the fact that the *Enteromorpha* was exposed to the air except at high tide, and the smaller fronds, growing in tufts and more closely adhering to the piles, retained the moisture longer and were consequently more favorable for growth.

When viewed with the ordinary low powers of a microscope, *Chlorocystis* appears a bright green color, usually of exactly the same shade as the *Enteromorpha* cells, but is not easily mistaken for them. If the cells of the host plant are dividing to form branch-like outgrowths and project above the surrounding tissue, there is a slight resemblance to the young

Chlorocystis, but a careful examination will at once reveal the difference.

In shape *Chlorocystis Cohnii* is usually spherical, although it may be slightly elliptical. It measures from 16–26 $\mu$  in the mature condition. Even though the plants are frequently crowded together in irregular masses, they never lose their characteristic outline.

The question as to the degree to which *Chlorocystis* may infest the host plant is one upon which my observations do not agree with those made previously. Both Lagerheim (12) and de Wildeman (4) describe the plant as being completely surrounded by the cells of the host, except for a small colorless portion which projects beyond the surface, through which the zoospores escape. Wright in his original description conveys the same idea, although he says, "sometimes zoospores attach themselves in such quantities to *Schizonema* that there is no room to force themselves into the frond," and at such times they are said to show but little evidence of penetrating the host. *Chlorocystis sarcophyci* (16) is described as being completely embedded within the tissue of *Sarcophycus*. As may be seen from *fig. 2*, the plants as I found them were not always included within the host, but were quite as often merely attached to the surface of the *Enteromorpha*. While, as will be described later, the zoospores upon germination may send out processes which penetrate between the *Enteromorpha* cells and during further development may be more or less surrounded by these cells, the fact remains that many of the plants pass their entire existence without having at any time been within the tissues of the host. The crowded condition of which Wright speaks is not necessary to bring this about, for the epiphytic habit is just as apt to occur among single individuals entirely separated from one another, as when they are grouped together. Even when the lower half of the *Chlorocystis* cell is below the *Enteromorpha* (*fig. 5, b*), a firm but gentle pressure will usually free it, leaving a round clear space where it has crowded the host cells apart. At no time were cells observed completely covered by the *Enteromorpha*,

and the impossibility of such an occurrence is easily understood when we remember that the single layer of *Enteromorpha* cells is frequently less than half the size of the *Chlorocystis*, and that they could never any more than surround the alga in a very superficial way. When the host is a plant made up of a mass of tissue, it may be that *Chlorocystis* assumes a true endophytic habit, but in *Enteromorpha* it certainly does not seem possible. Occasionally zoospores get between the tubular frond of the host through some accidental opening and there develop into normal *Chlorocystis* cells, just as they would on the surface of the plant, but this can hardly be called endophytic.

There is no evidence that the *Enteromorpha* is inconvenienced in any serious way by the presence of the *Chlorocystis*. Even when the fronds were almost completely covered, the cells of the host plant retained their normal appearance and seemed to be capable of carrying on all their functions. *Chlorocystis sarcophyci*, according to Miss Whitting (16), exerts a direct influence upon the surrounding tissue, "causing at first a swelling and loosening of the tissue, and finally complete disintegration of the cells," but nothing of the kind takes place with *Chlorocystis Cohnii*. The only benefit the *Chlorocystis* seems to derive from its host is that of a convenient and easy place of attachment, the condition well expressed by the German term *Raumparasitismus*. The algæ upon which *Chlorocystis Cohnii* have been found are quite various. *Enteromorpha* and *Urospora* among the Chlorophyceæ; *Polysiphonia* among the Rhodophyceæ, *Ascophyllum* among the Phæophyceæ, and *Navicula* (*Schizonema*) among the Diatomaceæ have all been recorded as host-plants for this form. The hydrozoan, *Campanularia*, and the infusorian, *Vaginicola*, have also been found with *Chlorocystis* growing upon them, and it seems not unlikely that the peculiar condition described as the abnormal fruit of *Calothrix confervicola* by Harvey (7) and the abnormal cells of *Prasiola leprosa* figured by Jessen (8) may likewise be due to this alga.

*Chlorocystis* contains a single large chromatophore, which, according to Reinhardt (14), and Wille in Engler and Prantl (5),

is said to lie always upon but one side of the cell. This is not true, however, and it was only after a considerable number of specimens were examined that the "one-sided" chromatophore was observed. It so happened that all the specimens which were first found showed a chromatophore completely lining the cell wall, as indicated in *figs. 1, 2, and 4*. Later, however, examples were found more nearly resembling Reinhardt's figures (*figs. 3, 5a*); but while this condition frequently occurs, it can no more be considered characteristic than when the whole cell is lined. A large and easily discernible pyrenoid lies near the surface of the cell and can be followed through all the subsequent divisions of the chromatophore (*figs. 6, 7*). Material killed in picric acid and stained for some time in 2 per cent. acid fuchsin brought out the pyrenoids well, although Flemming's fluid with iron-alum-haematoxylin gave perhaps more satisfactory results. The chromatophore usually forms a definite dome-like thickening where it surrounds the pyrenoid, and this may extend into the cell in the way shown in *fig. 5a*. When the chromatophore does not entirely line the wall, it radiates from the pyrenoid in irregular bands or ribbons, and these frequently do not pass more than half way round the cell (*fig. 5a*). This is the condition which Reinhardt figures and which he considered a generic characteristic. It was thought for a time that cells in which the chromatophore formed a complete lining might represent a condition subsequent to the formation of zoospores and not really be the adult *Chlorocystis*. Cultures in a Van Tieghem cell did not give much information on this subject, for although zoospores would be formed and escape, they did not develop to anywhere near maturity. This was probably due to an insufficient supply of oxygen, for when cultures were made in Ward cells, or simply under large cover glasses kept in a moist chamber, the zoospores could be watched from the time they escaped until they attained their full development. By this means it was settled beyond a doubt that in the great majority of cases the chromatophore entirely lined the cell from the beginning, and that it was a perfectly normal arrangement throughout all the vegetative stages of the plant.

Near the center of the cell is a well-defined nucleus, from which the protoplasm radiates in fine strands. There are usually several small non-contractile vacuoles present and the whole cell contents is often quite granular.

Two sizes of zoospores are formed. The larger ones, which are spherical, measure  $6-7\ \mu$  in diameter (*figs. 10, 12*), while the smaller are only  $2.6-3.5\ \mu$  and are somewhat pyriform in outline (*figs. 11, 13*). The method of the formation of the zoospores is identical in both cases, except that there are more successive divisions in the formation of the smaller spores, thus producing a greater number. In the original description of the genus by Wright, it is stated that the zoospores are formed in a very few hours by free cell formation. By Reinhardt (14) this simultaneous formation is considered one of the points of distinction between *Chlorocystis* and *Chlorocytrium*. De Toni also uses this distinction to separate the two genera. That the zoospores in *Chlorocystis* are formed by free cell formation is undoubtedly wrong, and all of my observations go to substantiate those of Lagerheim (12) and de Wildeman (4), who both state that the spores are formed by successive divisions. All stages in this process may be observed (*figs. 6, 7, 9*), and nothing comparable to the description by Wright has ever been seen. Also the statement made by the discoverer of the genus that the zoospores are at first colorless and that the protoplasm seemed to project itself to one pole and there form a single cilium is not borne out by my observations. Both kinds of zoospores have each four cilia, with a single chromatophore lining the base of the cell. In the large zoospores the pyrenoid is easily made out, and in the hyaline end of both the large and small spores is found a lenticular or spherical red spot.

When the zoospores are fully formed and ready to escape, a circular piece about  $10\ \mu$  in diameter is cut out from the top or outer side of the cell. This may be entirely loosened, or frequently it simply turns back, remaining attached at one side (*fig. 11*) very much as in some of the *Chytridineæ*. It seems probable that the zoospores do not always escape in this manner,



for all the other observers of this plant have spoken of a colorless neck which projects beyond the surface of the cell and through which the zoospores make their way. Wright, however, has said to Miss Whitting (16) that when he found *Chlorocystis Cohnii* developed in the interior of tissue, the cells were sometimes quite globular. Certainly the figures of Lagerheim (12) and Reinhardt (14) do not correspond to the description of a plant possessing such a protuberance. If the *Chlorochytrium inclusum* of Kjellmann (9) is finally to find a place within the genus *Chlorocystis*, as has been suggested, we have still another example of a form without the neck-like protuberance. After the examination of dried specimens of *Chlorochytrium inclusum* Kjell. I am not inclined to think that it is a *Chlorocystis*; although the published figures are strongly suggestive of that genus. It is certain that at no time, among the hundreds of specimens of *Chlorocystis* which were examined, was there anything that resembled a colorless protuberance. It may be that the varying habitat has something to do with the difference in aspect which this alga often presents; at any rate it seems probable that the presence or absence of a colorless tubular portion through which the zoospores may escape is not of much importance.

When the zoospores are liberated they swim about for a length of time varying from a few minutes to two hours. No difference was discernible in the rate or length of activity of the two kinds of spores. In almost every case the spores escaped perfectly free and independently of each other, but in a very few instances it appeared as though they might have been enclosed in a delicate membrane as in *Chlorochytrium*. If there was such a membrane it must have been very frail and was suggested rather by the arrangement of the zoospores than by any actual observation. It always seemed to break up before any reagent could be added to demonstrate it, and it is quite possible that nothing of the kind exists. Such a membrane enclosing the zoospores could not be of any significance from a systematic standpoint, for even in forms where it occurs frequently, there are conditions which bring about its total disappearance.

Material in the laboratory showed the time for the escape of the zoospores to be usually from seven to ten o'clock in the morning. This probably varies with the changing conditions at the seashore, and since the alga was submerged for only a few hours twice a day, it seems likely that the time of zoospore discharge varies with the tides. Efforts to establish this fact were unavailing. Observations made during the night were likewise without result. Perfect aeration was found to be conducive to the formation and discharge of large numbers of zoospores.

The existence of two kinds of zoospores and the fact that conjugation takes place in certain closely related genera would naturally lead to the supposition that something of the same kind occurs in *Chlorocystis*. De Wildeman (4) quotes Lagerheim as having observed copulation, but I am unable to find such a statement in any of Lagerheim's papers. He does mention having seen two kinds of zoospores, and considers it probable that the larger spore is formed by conjugation, but I think does not claim to have seen the process. From my own observations I can say that it is certain the larger zoospores are not formed by conjugation, and that it is possible for both sizes of zoospores to develop into new plants without any fusion. This point was carefully investigated by means of Van Tieghem cell and other cultures, and the zoospores were observed during their escape and final coming to rest. There was at no time any appearance of conjugation, and the development of the spores, whether of the large or small variety, was always the same, the cells produced being similar in every particular to the characteristic adult plants. It may be that under different physiological conditions conjugation might occur, but at the present time no light can be thrown upon that point.

When a zoospore comes to rest upon the surface of the host-plant, its cilia disappear and a thin gelatinous wall is formed around it. The red spot is lost to view and the pyrenoid becomes more prominent. If the zoospore is to develop within the host instead of merely attaching itself to the surface, a short

colorless neck is pushed out, and this penetrates the Entermorpha frond between its cells and pushes them apart. When an entrance has been gained in this manner, the neck widens until the whole cell appears funnel-shaped, and this, after further growth, assumes its mature spherical condition. In a few instances zoospores were observed which had germinated without having come in contact with the host-plant, and it is an interesting fact that some of these sent out colorless tubes of a considerable length (*fig. 8*). These were all found in cultures of various kinds and may have been due to some unknown abnormal condition.

In the first published account of this plant, the zoospores were described as escaping from the mother cell without possessing any color. These colorless zoospores developed into colorless plants which remained so until they had nearly reached adult size when the protoplasm commenced to develop "green cromules." "These cromules," says Wright (16), "arise as minute points along the inner surface of the cell wall from whence they radiate to the nucleus giving the appearance of a number of necklaces hung in loops." Although I looked carefully for some such condition in my material I was unable to observe anything abnormal or unusual. The green zoospores gradually developed into mature green plants with definite chromatophores as described.

Resting spores were observed in material that had been kept in the laboratory for some time and had been allowed to dry up partially. They are formed by the thickening of the wall of the mature plant and the contents rolling itself into a solid mass of irregular outline. The spore thus becomes of a darker green shade, and the pyrenoid is lost in the increased density of the cell contents.

It will be seen from the foregoing that a number of points with regard to the structure and development of *Chlorocystis Cohnii* which have been considered by former investigators as characteristic can no longer remain as such. The habit of the plant is variable, and it certainly cannot be regarded as a universal endophyte. The chromatophore is quite as apt to line

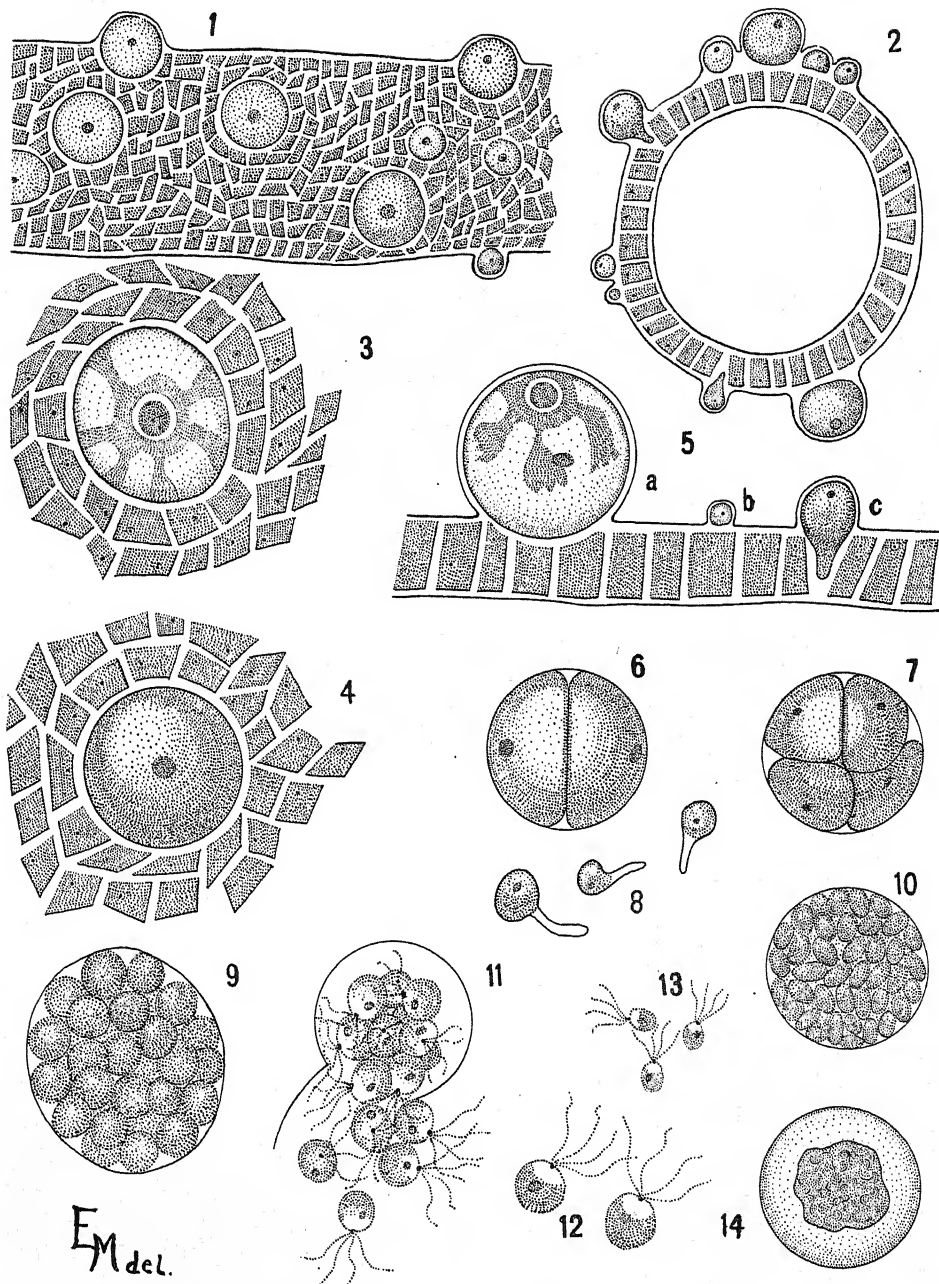
the cell wall as to be confined to one side and the radiate arrangement of the coloring matter may or may not occur. The method of zoospore formation is certainly the same as described by Klebs (10) for *Chlorochytrium Lemnæ*, namely by successive division. Even the manner in which the zoospores escape seems to vary, and the presence or absence of the colorless tube is of but little consequence.

It may be questioned whether or not the material found at Lynn really was *Chlorocystis*, since it fails to agree with any published account. Certainly much the easier way would be to regard it as a new genus. But while the plants found do not agree with the keys in de Toni, and Engler and Prantl, or with any other published account, the points of resemblance are very marked when all the literature is considered as a whole and the various generic characteristics correlated. The few papers on the subject are strangely at variance, and the figures in at least one case do not agree at all with the accompanying description, nor with the specimens distributed by the author. Consequently it seems a case where we are justified in disregarding certain published accounts and in considering that the form above described is really what Wright and Reinhardt meant for the plant *Chlorocystis Cohnii*.

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#### EXPLANATION OF PLATE X.

All the figures are from ink drawings sketched in with an Abbé camera. In the reproduction they are reduced one fourth. *Figs. 1* and *2* are drawn with a Leitz  $\frac{1}{8}$ , oc. 3. All the others with a Leitz  $\frac{1}{8}$  (oil), oc. 3. The magnifications given are the original ones before reduction and allow for projection.

#### *Chlorocystis Cohnii* (Wright) Reinhardt.

FIG. 1. General habit showing appearance of cells in Enteromorpha frond.  $\times 280$ .

FIG. 2. Section through Enteromorpha showing relation of Chlorocystis to its host.  $\times 280$ .

FIG. 3. Surface view of single cell. "One-sided" chromatophore with pyrenoid and radiate arrangement.  $\times 830$ .

FIG. 4. Surface view of single cell with chromatophore lining entire wall.  $\times 830$ .

FIG. 5. Side view; *a*, showing arrangement of radiating "one-sided" chromatophore; *b*; zoospore which has come to rest directly over Enteromorpha

cell; *c*, developing zoospore with projecting neck penetrating between *Enteromorpha* cells.  $\times 830$ .

FIGS. 6, 7. Surface views of first two stages in the formation of zoospores.  $\times 830$ .

FIG. 8. Germinating zoospores in cultures not in contact with host.  $\times 830$ .

FIGS. 9, 10. Sporangia of large and small zoospores respectively.  $\times 830$ .

FIG. 11. Sporangium of large zoospores showing method of discharge.  $\times 830$ .

FIGS. 12, 13. Large and small zoospores.  $\times 830$ .

FIG. 14. Resting spore.



## BRIEFER ARTICLES.

### NOTE ON THE MECHANICS OF THE SEED-BURYING AWNS OF *STIPA AVENACEA*.

(WITH FIVE FIGURES)

IN the parts of many plants where hygroscopic movements take place in dead tissues, the cause is found in thick-walled mechanical cells of peculiar structure or varying chemical composition.

The ripe and dry awn of *Stipa avenacea*, holding the seed at the lower end, is strongly twisted over half its length next to the seed in a direction opposite to the movement of watch hands. The remainder of the awn, not having the spiral structure of the former, is not twisted, but is bent at an angle to the body of the awn, thus furnishing a brace or support when the seed begins its boring motion, driven by the alternate twisting and untwisting of the dry or wet awn. Little barbules on the upper part of the awn directed away from the seed, assist its progress forward while preventing any backward movement.

The seed is tipped with a short, sharp point, slightly curved, the better to lead the way into the ground. Stiff hair-like barbs on the lower portion of the seed hold it in the ground when once started. The onward motion is still farther assisted by the increased length of the awn when wet, which amounts, by actual measurement, to 20 per cent. of the whole length. On drying a corresponding withdrawal of the seed is prevented by the barbs. So the alternate wetting and drying of the awn serves the twofold purpose of moving the seed about (although this is, no doubt, more commonly accomplished by being fastened with its appendages to some moving object) and placing it in a favorable position in the earth for germination.

The awns of *Avena* act much more promptly than those of *Stipa*; the latter take several minutes to straighten out in water, while one of *Avena barbata*, thrown on water, made five or six turns in twice as many seconds. The moist awn straightens out completely. When placed in caustic potash or other macerating fluid it twists with watch

hands almost as strongly as it does in the opposite direction when dried.

The minute structure of the awn furnishes an explanation of the phenomena here described. A thorough investigation of this subject was made by A. Zimmerman<sup>1</sup> whose purpose was to gain a more accurate insight into the torsion mechanism of the awns of wild grasses. He gives Hildebrand credit for first attempting to explain mechanically the hygroscopic torsion. His explanation was considered incorrect, and was not in accordance with the views of Nageli and Schwendener, who held that the seat of the mechanism is in the individual cell. Francis Darwin afterward confirmed this view. Zimmermann found that in the awn of *Avena sterilis* (with which, he says, *Stipa pennata* agrees in all essentials), the twisting power is confined solely to the outer row of cells whose structure shows a spiral arrangement. Both the arrangement of the pits in the walls of these cells and the appearance of the material in polarized light evince their spiral structure. The cells within this outer row have no tendency to twist, but the author thinks they assist the general movement by their contraction on drying. While so far little account had been taken of the micellar arrangement in the cells, the explanation of the mechanism was thus carried back to the molecular structure of the cells.

The present observations confirm those of others in locating the cause of the twisting of the awn in the individual cells *and show that not only a layer of cells but all of the mechanical cells are active in bringing about this result.*

As is well known, the twisted portion of the awn is composed principally of sclerenchyma cells with a fibro-vascular bundle in the center and a band of chlorophyll bearing tissue on each side (*fig. 1*). The latter, however, has nothing to do with the torsion. A striking peculiarity of the mechanical cells is the narrowness and eccentricity of their lumina; furthermore, this eccentricity in all the cells is alike, so that the lumina lie nearest the center of the awn (see *figs. 1 and 3*).

Strong Schultz's solution shows that the material immediately around each lumen is still very much like cellulose; that it swells and contracts more than the outer and denser layers of the cell wall is evident, since the surface of a cross section of a single cell in caustic potash is convex, but when washed and dried is concave.

<sup>1</sup>Jahrbücher für wissenschaftl. Bot. 7: 542.

As before stated, Zimmermann inferred the spiral arrangement of the material in the walls of the outer cells from the direction of the pits, and from the appearance in polarized light; but according to him no one had succeeded in bringing out clearly any striation.

After treatment with strong caustic potash and then with dilute glycerine, I found such striations quite well marked, passing obliquely

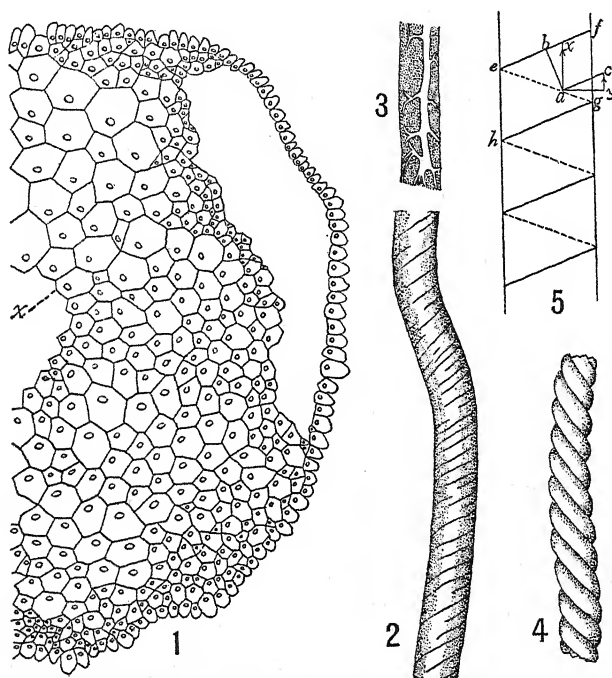


FIG. 1. Cross section of half an awn showing the disposition of the mechanical cells.—FIG. 2. An isolated cell (dry) showing the spiral arrangement of the material in normal condition.—FIG. 3. An optical longitudinal section of one of the cells (*x*) shown in figure 2.—FIG. 4. One of the cells after soaking in a macerating agent.—FIG. 5. Diagram showing the resultant of forces that may cause torsion in the mechanical cells.—FIGS. 1, 3, and 4 are enlarged 115 times.

across the cell (*fig. 2*). When seen through the cell in the opposite wall, they pass in opposite direction to those in the wall on the nearer side. This makes patent the spiral structure of the cell wall, denser (more highly refractive) layers alternating with layers which are less dense.

That the outer layer of cells is not, in this case at least, exclusively instrumental in effecting torsion, was proved by scraping off the outer two thirds of a wet awn, when the remaining central portion, on drying, was seen to twist as perfectly as the intact awn. Another evidence of this fact is, that after maceration, the largest cells, which belong to the middle portion of the awn, are found twisted quite as much as the smaller outside cells (*fig. 4*). This also agrees with Darwin's observations, quoted by Zimmermann (*l. c.*, p. 551).

There can be no doubt, then, that the mechanism is in the individual cells, and in the inner as well as the outer; and we have as an explanation of the hygroscopic torsion, thick-walled mechanical cells, each with very small eccentric lumen which is surrounded by a layer of cellulose-like material, the molecular structure of which is spiral.

There are two causes present, either of which may under favorable circumstances produce torsion:

*First*, the mechanical cell may be considered as a hollow cylinder whose walls are made up of material in layers of alternating density. In the diagram (*fig. 5*) let *fe* and *hg* represent dense layers of material while the less dense are the layers between. When dry, the cell is twisted with watch hands. Water enters first into the less dense layers forcing the micellæ in all directions. Two of these forces are principally concerned here. The one, *ab*, acting at right angles to the spiral plane of more dense material, may be resolved into its two components *ax* and *xb*. One of these acts tangentially and tends simply to increase the diameter of the cell; the other moves the spiral plane in such a way as to increase the angle it makes with the axis of the cell, producing, in the wall of the cell toward us, motion from right to left. The other force, *ac*, may be resolved into its components, *ay* and *cy*, the first of which again merely tends to increase the diameter of the cell, and the second, acting nearly parallel to the component *ax*, will strengthen it. In the opposite wall of the cell the same forces will be found to produce the same result, but when seen through the cell, the direction of motion will be just opposite to that in the wall on this side. There can be but one result from the action of such forces — the two forces on opposite sides of a cell, acting in opposite directions about its center, will produce torsion.

*Second*, the eccentric position of the cellulose-like material about the lumen of the cell throws the center of the more dense material to one side of its axis, so that the dry cell on imbibing water will curve

with the denser material on the concave side; at least this would ordinarily take place. But such a bend in one plane is changed to a twist whenever the proper forces are present. In the case before us, we have not only the proper forces to cause a twist but also to give the motion constancy of direction, *i. e.*, with watch hands. These forces are found in the spiral arrangement of the material. In this case we should expect a waving or serpentine bend rather than a close twist. The fact that many cells are found, after applying reagents, in all stages from a beginning bend in one plane to a wavy twist, leads to the conclusion that this is, perhaps, the principal force of torsion when the lumen is very eccentric.

It is probable that both these causes act in conjunction to produce the generally resulting perfect torsion. — L. MURBACH, *Central High School, Detroit, Mich.*

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#### SOME NEW SPECIES OF WYOMING PLANTS.

**Silene Tetonensis.**—Stems several, somewhat cespitose from a multicapital caudex, 10–25<sup>cm</sup> high, 1–7-flowered: minutely pubescent throughout, glandular above and often throughout, the leaves often glabrous except on the margins: leaves connate at base and sheathing by somewhat scarious membranes, the petioles often sparsely ciliate; the radical long-petioled, linear or narrowly oblanceolate, 2–8<sup>cm</sup> long, 2–6<sup>mm</sup> wide; the cauline linear or the lowest pair narrowly oblanceolate: calyx obovoid, 7–10<sup>mm</sup> long, with 10 purplish nerves, these anastomosing somewhat near the summit, 5-toothed, the teeth rotund or rhomboid-triangular, obtuse with very broad membranous margins: petals 9–12<sup>mm</sup> long, greenish-white or rose-color, more or less exserted; the claw 3<sup>mm</sup> broad, spatulate, with the margins entire or bluntly toothed near the summit, not at all auricled, 3-nerved, the nerves branched and anastomosing in the limb; this 3<sup>mm</sup> long, as broad as the claw or generally a little narrower, with no lateral lobes, emarginate or cleft to the middle, the lobes entire and rounded, the appendages much broader than long and bluntly toothed: stamens nearly as long as the claws of the petals, the filaments glabrous; styles 3, 1<sup>mm</sup> long: carpophore very short.

Related to the western *S. Watsoni*, but is readily distinguished from that by its broader radical leaves and very different petals.

The type is no. 6521 from the high grassy slopes of the Teton mountains, August 16, 1899; also no. 6684 from Dunraven peak in the Yellowstone park, August 27.

**Heuchera saxicola.**—Scape, petioles, and inflorescence villous and viscid-glandular, the leaves sparsely so: leaves oval to rotund, truncate or subcordate at base, doubly and incisely crenate with obtusish teeth, 2–4<sup>cm</sup> long, on petioles 4–9<sup>cm</sup> long: scapes slender, 2–4<sup>dm</sup> high, with two diminutive bract-like leaves near the summit: spike simple, often interrupted below, 3–6<sup>cm</sup> long; bracts ovate in outline, acuminate, 3-cleft to nearly entire and rhomboid, very ciliate on the margin, frequently tinged with violet, 6–8<sup>mm</sup> long: calyx campanulate, about 7<sup>mm</sup> long, very pubescent and glandular, villous at base, divided to about the middle; the tube adnate to the ovaries for slightly over half its length; the lobes unequal in size, ovate or oval, obtuse, white and petaloid: petals wanting: filaments from triangular to subulate, 1<sup>mm</sup> or less in length: capsule ovoid: seeds oval, hispid, brown.

*H. saxicola* is a near relative of *H. ovalifolia* Nutt., to which it is apparently referred in the recent published *Catalogue of the Flora of Montana*, for a specimen, collected by Rydberg and Bessey in the Spanish basin, belongs to this species. Referring to the original description, we find that *H. ovalifolia* is "wholly destitute of villous hairs," a negative character which at once excludes the plant here described. *H. ovalifolia* is originally from the region of the Columbia river, and is quite fully described in Howell's *Flora of Northwest America*. As here characterized, it has lanceolate and lacinate bracts, while in ours they are ovate in outline and either rhomboid or 3-cleft. The calyx is also described as tubular and with lanceolate lobes. In *H. saxicola*, on the other hand, the calyx is rather campanulate and the lobes broader.

Type, no. 5687, Undine falls in Yellowstone park, July 6, 1899; also, no. 2822, Little goose creek cañon, July 17, 1896.

**Saxifraga cognata.**—Cespitose, the numerous short, erect stems (1–3<sup>cm</sup> long) forming dense cushions, the lower part of the stems covered with old leaves: leaves imbricated, oblong-linear to lanceolate, cuspidate, 5–10<sup>mm</sup> long, hispid ciliate on the margins, otherwise glabrous: flowering stems slender, about 1<sup>dm</sup> high, sparsely covered with short purple-tipped hairs, as also the pedicels of the flowers, bearing several linear oblong leaves, these 3–5<sup>mm</sup> long and naked on the margins, or nearly so: inflorescence cymose, 5–7-flowered, seldom 9: calyx divided two thirds to three fourths; the lobes ovate, obtuse, or acutish, indistinctly 3-nerved, somewhat less than 2<sup>mm</sup> long: petals

elliptic, 4-5<sup>mm</sup> long, white, tinged with yellow toward the base, the upper half with purple dots, 3-nerved, the lateral ones arising near the base and extending to near the apex: ovaries united their whole length, the slender, conical styles more or less divergent.

This includes all of the so-called *S. bronchialis* of the Rocky mountains, and perhaps also of the mountains of the Pacific states. *S. bronchialis*, which is originally from Siberia, is everywhere described as having lanceolate sepals and orange-dotted petals, while our plant has ovate sepals, and petals quite prominently dotted with purple.

No. 5551, from Golden gate in the Yellowstone park, June 28, 1899, may be taken as the type of this species.

**Ribes saximontanum.**—A low shrub; spreading stems about 6<sup>dm</sup> long, more or less bristly, with straw-colored and shreddy bark; infra-axillary spines three together, stout, 8-12<sup>mm</sup> long: leaves orbicular or broader, truncate at base, 3-lobed half way to the base, the two lateral lobes again somewhat 2-lobed, the lobes with obtuse or acutish teeth, very finely pubescent on both sides or glabrate, 6-20<sup>mm</sup> broad: flowers 1-3, axillary, about 1<sup>cm</sup> long: calyx cylindraceous, glabrous, white tinged with violet: the tube 2<sup>mm</sup> broad or less, 4<sup>mm</sup> long, villous within; the lobes oblong, minutely toothed at the rounded summit, slightly shorter than the tube: petals cuneate-spatulate, toothed at the broad summit, about 2<sup>mm</sup> long: stamens included, the anthers oblong and obtuse: style divided half way to the base, villous: berry globose, smooth, reddish-purple, 6-10<sup>mm</sup> in diameter.

An excellent species, quite different from its southern relative *R. leptanthum*, from which it is readily distinguished by its bristly stems, glabrous whitish calyx, and divided villous style. It is an inhabitant of high, open slopes.

Type. no. 5542. Golden gate in Yellowstone park, June 28, 1899; also no. 695, Garfield peak, July 29, 1894, by *Aven Nelson*.

**Rosa grosse-serrata.**—Much branched, 6-18<sup>dm</sup> high, with occasional short prickles (less than 5<sup>mm</sup> long) or wholly unarmed, the branches purple and somewhat glaucous; stipules usually broad, 12-20<sup>mm</sup> long, entire or minutely glandular-toothed, the free ends triangular ovate, acute, or acuminate, usually minutely resinous dotted and finely pubescent: leaves 5-7-foliolate; the leaflets nearly sessile, the terminal petiolulate, elliptic to narrowly and cuneately obovate, sharply and coarsely serrate for about two thirds their length, 2-4<sup>cm</sup> long, glabrous above, sparsely and very finely pubescent and minutely resinous dotted (under

a strong lens) beneath as also the rachis: flowers in clusters of two to four at the ends of the branches or solitary: sepals entire, linear-lanceolate, attenuated, the tips only slightly dilated, sparsely pubescent on the back and occasionally hispid-glandular along the margins; petals unknown; fruit globose, perfectly smooth, about 12<sup>mm</sup> in diameter.

Apparently intermediate between *R. pisocarpa* and *R. Woodsii*. From the former it differs in its stouter branches, in the minutely resinous lower surfaces of the leaves, and in the larger and more coarsely toothed leaflets. From the latter one cannot so easily find characters by which to distinguish it, yet it is so different in appearance that its separation as a distinct species seems to be justifiable. The so-called *R. Woodsii* of the Rocky mountains is a much lower plant, and has leaflets only 2<sup>cm</sup> long.

The following collections of this species are at hand: no 1101, Boulder creek, August 27, 1894; no. 6787 (type), Madison river in the Yellowstone park, August 30, 1899; both by *Aven Nelson*.

**Lupinus ramosus.**—Stems several from a woody caudex, 2-4<sup>dm</sup> high, branched, with divaricate branches, these simple and terminating in short few-flowered racemes: stems and petioles with two kinds of pubescence, finely canescent and sparsely villous with spreading hairs: leaves 5-8-foliate, the lower half the length of the petioles, the upper equaling them; leaflets narrowly oblanceolate, obtuse to acute, usually mucronate, densely soft-silky on both sides, 2-4<sup>cm</sup> long, 5-9<sup>mm</sup> wide: racemes short-peduncled, 3-5<sup>cm</sup> in length, in fruit a little longer: bracts ovate or lanceolate, about 2<sup>mm</sup> long: flowers somewhat verticillate or scattered, about 1<sup>cm</sup> long; pedicels 2-3<sup>mm</sup> long, in fruit 5<sup>mm</sup>; calyx bracteolate, densely silky, as also the pedicels, the lower lip a little longer than the upper, which is slightly notched: vexillum very silky on the back, the central portion yellowish-white, otherwise pale blue or lilac; wings pale blue, slightly longer than the vexillum; keel light-colored, ciliate on the margin except at the very tip: pod silky, 3-5-seeded.

Characterized by its branching habit, short and few-flowered racemes, and the two kinds of pubescence.

The type, no. 6576, is from dry banks and benches on Snake river, Yellowstone park, August 20, 1899.

**LUPINUS HUMICOLA Tetonensis.**—Stems one or more from the same root, simple 3-6<sup>dm</sup> high, including the racemes, 3-5-leaved; leaflets acute or obtuse and mucronate, glabrous on the upper surface or



sparsely strigose, 5-9<sup>cm</sup> long, 8-18<sup>mm</sup> wide: raceme 4-12<sup>cm</sup> long, mostly only 4-5<sup>cm</sup>.

No. 6341, Teton mountains, August 16, 1899.

**Antennaria fusca.**—Loosely caespitose: stolons about 5<sup>cm</sup> long: stems slender, 12<sup>cm</sup> high or less: leaves canescent or tomentulose on both sides, the older ones becoming glabrous; the radical and those of the stolons spatulate, indistinctly mucronate, 15-22<sup>mm</sup> long; the cauline linear, 2-4<sup>mm</sup> wide, the lower somewhat broadened upwards and acute, the upper acuminate: heads 3-13, on short pedicels in close clusters or loosely corymbose, and the head or heads of the lowest pedicel, which is often 2-3<sup>cm</sup> long, usually overtopping the rest: involucre about 6<sup>mm</sup> high: bracts (pistillate) in about two series, the lower half bright green and sparsely woolly, the upper portion brown or greenish-brown, oblong, obtuse, more or less serrate.

In habit and general appearance the species here described would suggest *A. aprica* Greene, yet it is not even a very near ally of that. Its dark-colored bracts, slender stems, and the dull and light indument of the leaves easily separate it specifically from Professor Greene's species.

The type is no. 6356, growing on dry bottoms and in open woods on Lewis river, Yellowstone park, August 8, 1899.

**Antennaria oblancifolia.**—Caespitose: stolons very short: stems slender, 15<sup>cm</sup> high or less: radical leaves oblanceolate, those of the stolons narrowly so, acute, mucronate, about 2<sup>cm</sup> long, sparsely canescent to glabrous above, canescently tomentose below: cauline leaves linear or oblong-linear, the lower acute, the upper acuminate: heads 4-13, in close racemose or paniculate clusters: involucre (staminate) 4<sup>mm</sup> high, the herbaceous portion of the bracts sparsely woolly, the scarious portion oval, obtuse, brownish, or white.

This is near to *A. racemosa*, but it is so strikingly different from that in the size and outline of its leaves that it must stand as a distinct species.

It is represented by a single collection in which all the plants are staminate, secured on an open, once wooded slope, near Mammoth hot springs in the Yellowstone park, July 3, 1899, no. 5640.

**Gnaphalium thermale.**—Low, 1-2<sup>dm</sup> high, branched from the base, the stems more or less branched throughout or simple to near the summit: stems and leaves grayish woolly: radical leaves oblanceolate, about 35<sup>mm</sup> long, the cauline narrowly oblanceolate to linear, 1-3<sup>cm</sup> long: heads sessile in small glomerules terminating the branches:

involucres 4-5<sup>mm</sup> high: bracts dull white, from ovate in the outer to linear in the inner, obtuse or acutish, nearly all apiculate.

A northern ally of the Texan *G. Wrightii*.

Collected on the geyser formations of Norris geyser basin in the Yellowstone park, July 25, 1899, no. 6139.

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### SOME NEW NORTH AMERICAN MOSSES.

(WITH PLATE XI)

IN July 1898 the writer, in company with Mr. James Blake, made a vacation trip into northwestern Montana, during which we collected especially mosses and hepatics. The region visited is reached by the Great Northern railway, which we left at Belton, thirty miles east of Kalispell. Thence we made our way to the north end of lake McDonald, some twenty miles north, where we pitched our permanent camp. The region visited is especially interesting because of the several glaciers which nestle among the precipitous mountain peaks. We visited only one of these, Sperry glacier, at the base of which we found several European mosses heretofore not reported for the United States, as well as some new species closely related to certain alpine species of the old world. So far as determined the material collected includes 140 species of mosses and 20 species of hepatics. The publication of a full report is delayed for various reasons, and it is deemed desirable to publish here only the most important part of the report as far as prepared. A more detailed account of this region, botanically as well as geologically very interesting, may be found in the September number of *Bulletin* of the American Bureau of Geography.

*Dicranoweisia subcompacta* Card. et Thér., sp. nova.—Dense pulvinato-caespitosa. Caulis simplex vel parcissime ramosus, 6-8<sup>mm</sup> altus, dense foliosus. Folia madida suberecta, sicca crispatula, 1-1.5<sup>mm</sup> longa, oblongo-lanceolata, acuminata, subacuta vel obtusiuscula, superne, canaliculata, nervo basi attenuato usque ad apicem producto vel paululum infra evanido, marginibus inferne planis, superne inflexis, integerimis, cellulis irregulariter quadratis vel subrectangularibus, inferioribus laxioribus, juxta costam linearibus, alaribus distinctis, subinflat, fuscis. Caetera ignota.—*Plate XI*.

Very nearly allied to the European *D. compacta* Sch., from which it differs by the leaves being more narrowly acuminate and generally subacute, the cells of the areolation larger and with thinner walls, and chiefly by the costa narrower, attenuate below ( $16$  to  $25\mu$  broad; it is  $55\mu$  in *D. compacta*).—Along the trail from Holzinger's basin to the Rim.

**Barbula rufipila** Card. et Thér., sp. nova.—*B. aciphyllae* habitu et foliorum forma omnino similis, differt tantum cellulis duplo majoribus et magis distinctis (superioribus  $20$ – $30\mu$  in *B. rufipila*,  $12$ – $15\mu$  in *B. aciphylla*) piloque saepius minus denticulata interdum integro. Specimina sterilia.—*Plate XI*.

Avalanche basin; Holzinger's basin.

**FISSIDENS BRYOIDES GYMNAANDRUS** (Buse) R. Ruthe.—New to North America. Cardot det.—Shores of lake McDonald; Avalanche trail.

**Grimmia Holzingeri** Card. et Thér., sp. nova.—Minima, tenella, pulvinatula, obscure viridis, inferne fusca. Caulis erectus,  $4$ – $6^{\text{mm}}$  altus, parce ramosus, ramis interdum attenuatis, subflagellaceis. Folia conferta, minima,  $0.50$ – $0.70^{\text{mm}}$  longa,  $0.20$ – $0.35$  lata, madida erecta, sicca appressa, breviter ovato-oblonga, concava, omnia mutica obtuse acuminata, marginibus planis integris, costa canaliculata, usque ad apicem producta, basi  $28\mu$  lata, cellulis superioribus bistratosi, quadrato-subrotundatis, inferioribus unistratosi majoribus, lutescentibus, infimis oblongis vel sublinearibus, omnibus incrassatis. Caetera ignota.—*Plate XI*.

This very minute species, resembling in habit the small forms of *Andreaea petrophila*, is quite distinct from all the European and North American species of *Grimmia* with muticous leaves by the small size, and the shape and areolation of the leaves.—Base of Sperry glacier; Mt. Trilby.

**GRIMMIA MOLLIS** B.S.—This European alpine moss is reported from Greenland, and should be found at intermediate stations in Canada.—Base of Sperry glacier.

**GRIMMIA SUBSULCATA** Limpr. in Rabenh. Cryptog. Fl., Laubm. 757.—New to North America. Cardot det.—Mt. Trilby.

**WEBERA CARINATA** (Brid.). (*W. cucullata carinata* Husnot; *Bryum naviculare* Cardot).—New to North America. Cardot det.—Base of Sperry glacier.

**BRYUM ALPINUM** L., var. **denticulatum** Card. et Thér., n. var.—A forma typica differt habitu graciliore, foliis ovato-acuminatis,

brevioribus, marginibus parum revolutis, superne distincte sinuato-denticulatis, costaque longe ab apice dissoluta.—On the way from Holzinger's basin to the Rim.

*PSEUDOLESKEA RADICOSA* (Müll.) Lesq. & James.—This species was distributed as *P. rigescens* Lindb.: it is the *P. atrovirens* of European authors. Best det.—Holzinger's basin; Mt. Trilby.

*PSEUDOLESKEA DENUDATA* HOLZINGERI Best, in Bull. Torr. Bot. Club 27: 229. May 1900.—Holzinger's basin; Mt. Trilby; Avalanche basin.

*Hypnum Cardoti* Thér., sp. nova.—Polygamum, olivaceo-viride, molle, laxiuscule depresso-caespitosum. Caulis procumbens vel ascendens, irregulariter ramosus, 2–4<sup>mm</sup> longus. Folia remotiuscula, patulosquarrosa, interdum subsecunda, e basi constricta anguste decurrente late ovato-delloidea, subito in acumen angustum breviusculum recurvum protracta, circa 1.5<sup>mm</sup> longa et 0.75 lata, marginibus planis fere undique sinuato-denticulatis, costa simplici bifurcata vel gemella, crure longiore ad medium producto, cellulis laxiusculis linearibus subflexuosis, basilaribus brevioribus et latoribus, alaribus laxis majoribus subhyalinis. Folia perichaetialia externa ovato-lanceolata, breviter acuminata, subintegra, enervia, intima plicata, costata. Capsula in pedicello rubente valde flexuoso, circa 18<sup>mm</sup> longo, subhorizontalis, arcuata, operculo convexo apiculato.—*Plate XI.*

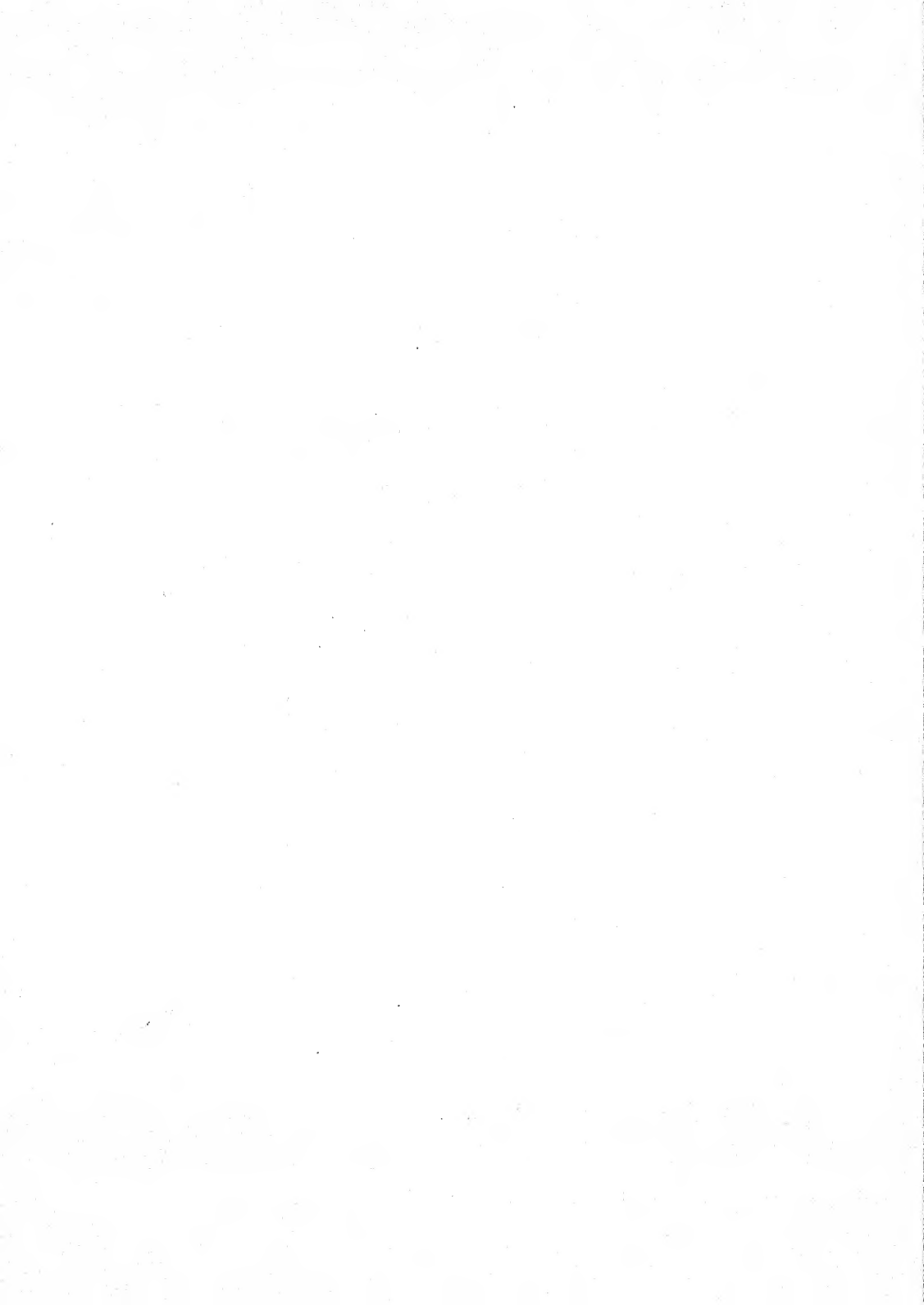
This species is near *H. stellatum* Schreb. and *H. polygamum* Sch. From the first it is at once distinguished by the polygamous inflorescence and the softer leaves with a shorter acumen and a looser areolation. The shape of the stem leaves and of the perichaetial leaves distinguishes it from the small forms of the second species.—Avalanche basin.

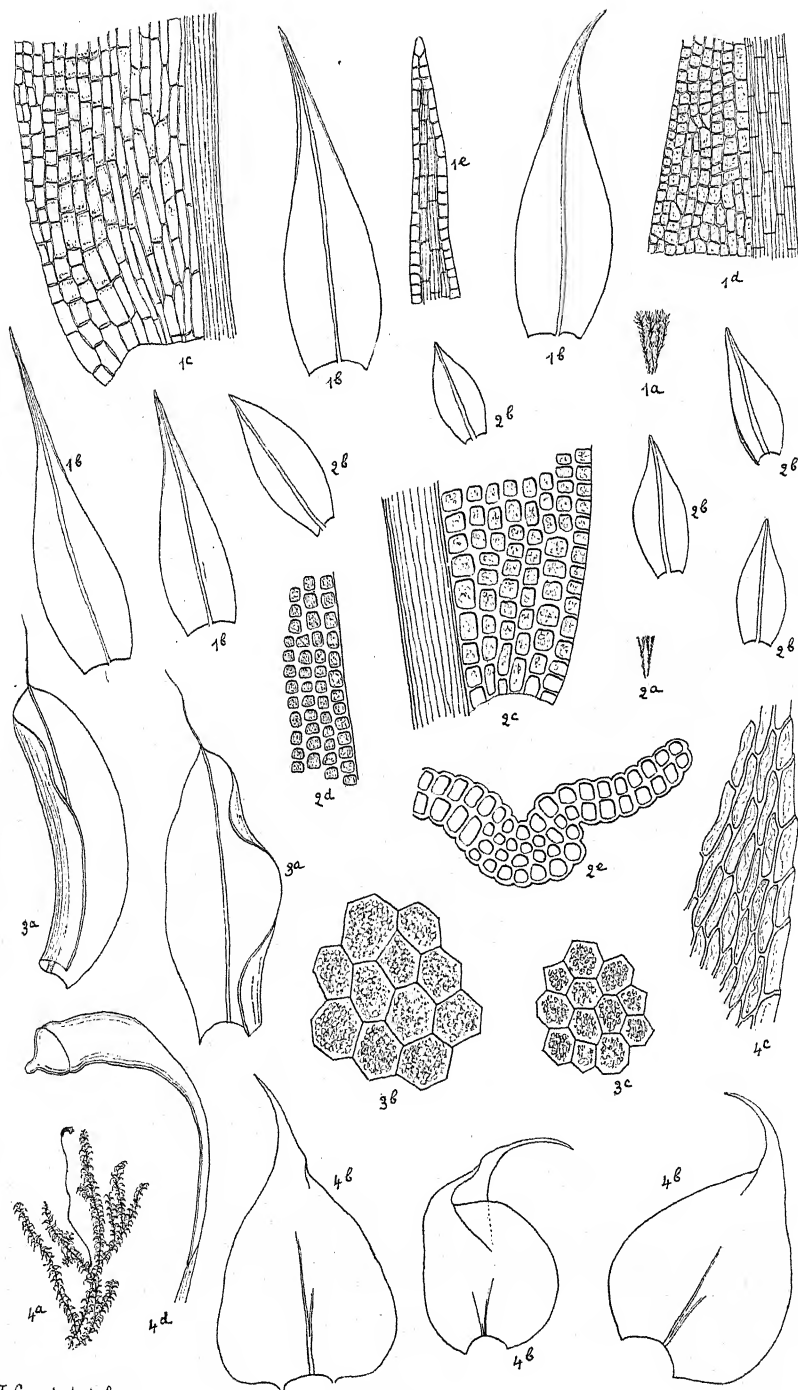
*HYPNUM FLUITANS* L., var. *BRACHYDICTYON* Ren. in Husnot Musc. Gall., forma *Holzingeri* Ren.—Voisin de la var. *brachydictyon* Renauld, n'en diffère que par le port plus grêle, la nervure plus étroite, et le tissu délicat. Dioïque ♀!

Cette var., essentiellement alpine, n'avait pas encore, je crois, été signalée en Amérique.

A cause de la brièveté des cellules médianes, on pourrait confondre cette forme avec *Hypnum aduncum* Hedw.; mais le passage brusque des cellules foliaires de la base aux cellules superficielles de la tige permet d'éviter la confusion.—Base of Sperry glacier.

*HYPNUM OCHRACEUM UNCINATUM* Milde.—A European alpine moss, new to North America. Renauld det. Holzinger's basin.





J. Cardot del.

HYPNUM UNCINATUM Hedw., var. SUBJULACRUM Sch., forma *Holzingeri* Ren.—Forme voisine de la forme *orthothecioides* Lindb.; en diffère par la couleur verte, les touffes compactes encombrées de terre à la base, l'acumen plus court denticulé et le tissu plus délicat, non épaissi.—Base of Sperry glacier.

Minor extensions of range will be noted in a fuller report on this collection.—JOHN M. HOLZINGER, *Winona, Minn.*

#### EXPLANATION OF PLATE XI.

(Nacht's objectives 3 and 6, oculars 1 and 3, with camera lucida. All drawings are reduced  $\frac{1}{4}$  in photo-engraving.)

1. *Dicranoweisia subcompacta*. *a*, entire plant, nat. size; *b*, *b*, *b*, *b*, leaves  $\times 32$ ; *c*, basal areolation  $\times 135$ ; *d*, marginal areolation in the middle  $\times 135$ ; *e*, point of a leaf  $\times 135$ .

2. *Grimmia Holzingeri*. *a*, entire plant, nat. size; *b*, *b*, *b*, *b*, *b*, leaves  $\times 32$ ; *c*, basal areolation  $\times 285$ ; *d*, marginal areolation in the middle  $\times 285$ ; *e*, transverse section of a leaf  $\times 285$ .

3. *Barbula rufifolia*. *a*, *a*, leaves  $\times 13$ ; *b*, areolation in the upper part  $\times 285$ ; *c*, the same of *B. aciphylla* from a specimen of Styria  $\times 285$ .

4. *Hypnum Cardoti*. *a*, entire plant, nat. size; *b*, *b*, *b*, leaves  $\times 32$ ; *c*, marginal areolation in the middle  $\times 285$ ; *d*, capsule  $\times 13$ .

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#### NOTES OF TRAVEL. III.

##### RIO AND PETROPOLIS, BRAZIL.

To a professional traveler and to a botanist Rio de Janeiro and Petropolis have more to offer than any other easily accessible place in South America. Mr. Barbour Lathrop, with whom the writer has the pleasure of traveling as assistant, is familiar with many of the picturesque spots in the world, and even to him the region about Rio was a most agreeable surprise.

We visited together in 1897 the harbor of Sydney, Australia, which is most commonly compared with that of Rio, and were able to draw comparisons which are decidedly favorable to Rio.

Sydney harbor is long and, in comparison with that of Rio, narrow, with a great number of small coves separated by sharp points of land which jut out into the stream. These points of land, each side of the harbor, alternate with the coves opposite, like the teeth of a shark. These low and rounded hilly points are covered with Australian

scrub, which is composed of narrow-leaved acacias, eucalyptus, and numerous Myrtaceæ of a gray-green color. To those who tire of the gray-green of Italian olive groves, these narrow-leaved Myrtaceæ soon become monotonous, and the scanty shade shed by the sickle-shaped vertical leaves of the eucalyptus makes little in the landscape that is restful to the eye. There are scarcely any islands in Sydney harbor, and its principal charm to a traveler lies in these innumerable coves which on either hand pop into view from the steamer. They are often filled with shipping, and the shores are being rapidly denuded of their forest and scrub vegetation, though many quiet pretty views still remain. A botanist finds in the curious Proteaceæ, the most peculiar grass trees (*Xanthorrhoea*), and the endless variety of Myrtaceæ a host of forms which tickle his morphological sense with their novelty.

Rio harbor, on the other hand, is like a large inland lake, with numerous islands scattered through it, and surrounded by tall curiously rounded sugar-loaf hills. Islands, hills, and low-lying swampy shores are covered with a wealth of tropical vegetation quite as luxuriant as any to be seen directly under the equator. In place of the rounded gray-green of the hills of Sydney, Rio has dark imposing cliffs which reach above the low-lying clouds. Their deep ravines and valleys are a tangle of creepers, bright purple flowered melastomas, broad-leaved lilies, and innumerable epiphytic bromelias. Everything is steaming with moisture and the leaf tips are dripping with dew.

The islands in the harbor, though very picturesque, lack both cocoanut palms and coral reefs, two features which give to the islands of the south seas their peculiar charm. The former lack could easily be remedied, but the brilliant white coral reef would be difficult to supply.

Rio is the most picturesque city in South America. The Portuguese architecture is a great relief after the monotony of Spanish American cities, and no suburb in any city that I know is more charming than Botafogo, the residence portion of Rio. Each picturesque house, of yellow or pink stucco, trimmed with colored Moorish tiles and roofed with red tiling, is set in a half-neglected garden of tangled flowering creepers, bamboos, ficus trees, and foliage plants. Although not as well taken care of as the American gardens of Honolulu, they are much more numerous and picturesque. In fact, I do not believe there is a place in the world where such an array of picturesque gardens and houses can be seen as here in Rio.



The characteristic feature of Rio vegetation is made by the avenues of royal palms. Although there are avenues of this palm in Java, Hawaii, Jamaica, Trinidad, and Ceylon, in none are they really impressive. The first leaf-sheath in young plants is always objectionably prominent. The immense avenues of Rio are so tall that these leaf-sheaths are not noticeable. No more beautiful avenues of palms are imaginable than those of the Botanic Gardens and a double avenue near Botafogo.

The Botanic Garden lies an hour's train ride from the center of the city, in a locality unfortunately infested with yellow fever. Its most courteous director, Dr. Rodrigues, has made a special study of palms, and his collections are very tastefully arranged through the garden. Although containing many rare specimens, this collection does not compare in any respect with that in the gardens of Java or Ceylon. Owing to its situation so far from comfortable hotels and its lack of laboratory facilities, the garden will be a difficult place in which to prosecute botanical studies. In the winter months the danger from yellow fever would quite prohibit its being used except for a few hours a day, as no stranger who values his life risks living in the city, but spends his nights at least either at Petropolis, three and one half hours away, or at Tijuca, much nearer, but not so free from fever.

The charm of Rio, botanically, is in its surroundings. Petropolis, a city of twenty thousand inhabitants, lies at an altitude of three thousand feet among the mountains across the bay from Rio, and is reached by two hours of ferryboat and railway travel. During the season of yellow fever, from January to June, over two hundred passengers, mostly business men, make this trip twice a day, leaving Petropolis every morning and returning in the evening. As the danger from yellow fever is only incurred by exposure to the night air, Europeans live in perfect security by making their homes in Petropolis and never spending a night in Rio during its summer season.

The ride to Petropolis is richer in panoramic effect than any road of like length in the tropics, surpassing the famous railway journeys from La Guayra to Caracas in Venezuela, Colombo to Kandy in Ceylon, and from Padang to Padang Pandjang in west Sumatra. The vegetation near the shore of the harbor, through which the railway passes, resembles that of the dense swamps characteristic of the isthmus of Panama—a paradise for the collector of fresh water algæ, but infested with malaria. These low lands are covered with a tangled

jungle of ferns, convolvulus, bananas, and flowering lilies, enlivened by occasional characteristic melastomas, one blaze of dark purple flowers. As the train climbs the steep incline and the cogs of the miniature engine grasp the cog rail a panorama of increasing grandeur is unrolled. It comprises the whole harbor with its dark green islands and surrounding densely forest-clad mountains. Dark green cliffs, covered everywhere with masses of creeping plants, tower above the train, their tops concealed by low-lying clouds. At one point a deep narrow valley spreads out into the plain below and from the train one looks down upon square miles of tropical tree tops of various shades of green.

The evidences of intense insolation in the deep reds and bright yellows of the young foliage are few as compared with west Java, Sumatra, or Trinidad, and the occasional white foliage of a species of Araliaceæ remind one of the candle nut trees (*Aleurites*) in the Hawaiian landscapes. In the early morning a sea of clouds shuts out the view of the harbor and replaces it with a most curiously effective scene.

Rio is the largest city of Europeans within the tropics, and upon Petropolis, its fashionable suburb, has been expended, in beautiful villas and gardens, the wealth and taste of its most successful merchants. The climate is that delightful one characteristic of the high altitudes in this latitude. Cool, even cold, nights and bright warm days mark the winter, with occasional cloudy weather and frequent showers. In summer there is almost perpetual, sometimes uncomfortably warm, sunshine but with always cool nights. During our stay in May, corresponding to our autumn, the nights were cold, the mornings crisp and cool, and midday was never uncomfortably warm.

The forests immediately surrounding Petropolis are a second growth. Within easy walking distance, however, there are large stretches of virgin forest, with jungles in which climbing bamboos, tree ferns, purple flowering melastomas, begonias, occasional epiphytic orchids and phyllocactus, and an abundance of bromelias and lianas make up the undergrowth between the immense forest trees of Myrtaceæ and Ficus. The roadsides are lined with thickets of native raspberries, bearing attractive bright red but insipid fruit. The walls of the embankments are covered with mosses, filmy ferns, lichens, and liverworts, with occasional polypodium. The light gray trunks of the forest trees are spotted with bright red patches formed by a species of

lichen, and the branches of the trees are thick with fern, bromelia, and moss epiphytes, characteristic of moist tropical mountains. The sunny clearings in these forests are simply alive with brilliant butterflies, and here and there across the paths are files of the leaf cutting *Atta* ants, which Belt and Möller have so well described. Patches of *Cuphæa* by the roadside are especially subject to the attacks of these interesting leaf cutters, and their intelligent movements as they cut and carry the leaves and tender stems to their nests are perfectly fascinating and worthy a much more serious study than has so far been given them. Beaten paths cleared of all dead leaves and fragments of stone can be traced for many yards through the forest, leading to the openings of the nests, down which in endless streams the busy cutters tumble leaf fragments, which they have brought balanced between the spines of their thoraci.

A few inches beneath the surface of the ground these leaf fragments are masticated and molded into the sponge-like mushroom-bed upon which the fungus grows that forms the "*kohl rabi*," which is the necessary food of the species. A one sided warfare is waged against these attas by a powerful species of black ant, over half an inch long, and my friend and I watched with keen interest the slaughter of a dozen or so of the leaf cutting workers by a single one of these assassins, who killed every individual he found, as a terrier would a rat.

Although there are no laboratory facilities of any kind in Petropolis, its natural advantages for a botanist are exceptional. Proximity to the virgin forests, a climate in which one can work as comfortably as in New England, the most beautiful scenery imaginable, and a large and highly intellectual society of Europeans, make it a place without equal in the mountains of the tropics. Living in the hotels *en pension* would amount to about \$2 a day for all expenses. A bicycle could be used to excellent advantage in making excursions along the country roads, which are very good in the neighborhood of the town. Excursions to Cascatina, Itymarati, and the Organ mountains are full of the liveliest interest, and an abundance of material for morphological and biological studies can be easily gathered together.

The Corcovado is a tall mountain overlooking Rio, from which a view of unparalleled interest is obtainable and Tijuaca is a small village lying in the cul de sac between the hills behind it. The latter is surrounded by dense forests, which are penetrated by many miles of well kept wagon road, forming doubtless the most extensive natural

park of its kind in the tropics. Rio being the railway center of Brazil, excursions to the interior are made with comparative ease.

To any botanist who wishes to study tropical vegetation, and who has at the same time an eye for the beautiful, Petropolis and the other suburbs of Rio will prove beyond question the most attractive place in the world. As compared with the mountains of Java or Sumatra they are civilized, have a much more salubrious climate, and all the conveniences of modern civilized life. The south island of Hawaii or the south Pacific islands have no such stretches of virgin forest, nor such a flora or fauna to explore. Ceylon is hot and uncomfortable in comparison, and the mountains of Jamaica and Trinidad are uninhabited except by scattered planters.

It is the writer's belief that there is no other place in the world where such a combination of tropical vegetation, wonderful scenery, civilization, and cool equable climate can be found.—D. G. FAIRCHILD, *Port Said, Egypt*, September 21, 1899.

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#### ANOTHER NOTE ON THE FLOWER VISITS OF OLIGOTROPIC BEES.

SINCE the table in the BOTANICAL GAZETTE 28:36 was published, *Andrena arabis* has been found collecting pollen of *Cardamine rhomboidea*. In the second column, therefore, Cruciferae should be substituted for *Arabis laevigata*.

Of the three species of *Calliopsis* enumerated on page 28 of the same volume, one, *C. andreniformis*, is quite polytropic. Another, *C. coloradensis*, I think, is oligotropic and gets its pollen exclusively from Compositae. It collects pollen of *Boltonia asteroides* and *Coreopsis aristosa*.

A male of the third species was taken on August 23, 1897, on flowers of *Verbena stricta*. I did not care to describe it until I had found the female. July 15, 1899, the sexes were taken in copula on flowers of *Verbena urticifolia*, the females collecting the pollen. The males were also taken on *V. hastata*. In the mean time, the bee was taken in New Mexico on flowers of *V. macdougalii* by Miss Porter, and was described by Cockerell under the name of *Calliopsis verbenae*. This author says nothing about it collecting the pollen. The bee is evidently an oligotropic visitor of *Verbena*.—CHARLES ROBERTSON, *Carlinville, Illinois*.

## CURRENT LITERATURE.

### BOOK REVIEWS.

#### A practical garden book.

THE LATEST of the Garden Craft Series is a compact little book of 250 pages for amateur gardeners,<sup>1</sup> in which the subjects are arranged in cyclopedic fashion. This alphabetic arrangement is supposed to make unnecessary both table of contents and index, but the latter would have been useful for cross references.

The range of subjects treated is wide, as may be seen by the following, taken at random from "C": Cucumbers, Currants, Cuttings, Cutworms. A section from "F" reads as follows: Ferns, Fertilizers, Feverfew, Fig, Flowerbeds.

The aim has been to afford information on simple garden operations in the simplest possible form. The effort, so far as it has been carried out, has been fairly successful, and as a rule little fault can be found with the quality of the advice given, although under "Dahlia" the direction given to "place the roots in gentle heat" might easily lead the amateur into difficulties unless he is familiar with technical terms.

The information is mainly of local application, being adapted to central and northern latitudes. The book may prove of some value as a reference book to persons entirely ignorant of gardening operations in much the same way as an alphabetical list of common French or German phrases would be of value to the traveler in Europe.

*Garden making*, an earlier book of this series, is greatly superior, as it covers a wider field and treats in plain language the principles of the art of gardening, something absolutely essential for the beginner. It is extremely doubtful if a beginner, in consulting the present volume, would look up soils, cultivation, fertilizers, etc., before sowing his seeds. As a companion, possibly as a complement to *Garden making*, it will be useful to those who can buy many books, but, taken by itself as a garden guide, it is of comparatively little value. Indeed its publication leads one to suspect an effort to unload on the public a new book simply for the purpose of gain; an effort of publishers too often attempted and commonly successful. And Professor Bailey's name seems to be attached to this volume merely as a makeweight for this purpose. — F. W. CRANFIELD.

<sup>1</sup>HUNN, C. E., and BAILEY, L. H.: Amateurs practical garden book, containing the simplest directions for the growing of the commonest things about the house and garden. Pp. vi + 250. Illustrated. New York: The Macmillan Company. 1900. \$1.00.

### Three popular books.

POPULAR interest in plants is being cultivated recently by numerous books of high artistic merit and variable scientific quality. Several of them have been noticed in this journal, and three others now deserve attention, all of which stand high in their class. To interest the public in making intelligent observations is a most commendable purpose, but the temptation to arouse interest by bringing together doubtful facts and legends is very strong, and the effect is sometimes more than doubtful.

*Nature's garden*<sup>2</sup> is the title selected by Neltje Blanchan (a *nom de plume*, we understand, of Mrs. Doubleday). The book considers flowers from the standpoint of pollination by insects, and the best authors seem to have been consulted. Over five hundred flowers are represented, and are grouped according to color, "because it is believed that the novice, with no knowledge of botany whatever, can most readily identify the specimen found afield by this method, which has the added advantage of being the simpler one adopted by the higher insects ages before books were written." The classification by colors is as follows: blue to purple flowers, magenta to pink flowers, white and greenish flowers, yellow and orange flowers, red and indefinites. The book is on a higher plane than those which merely seek to lead the observer to a name, for the name is only an introduction to some real acquaintance. The illustrations deserve special commendation, for the half tones from photographs and the colored photographs made direct from nature are of exceptional merit. The former bring out details with that exquisite clearness which good photographs can obtain, while the latter represent the best results of color photography, which we must confess is frequently uncertain as yet as to shades. The book appeals to those interested in beautiful illustration, to those desiring a better acquaintance with flowering plants, and to those who are seeking on every hand help in organizing nature study.

ALICE LOUNSBERRY, the author of *A guide to the wild flowers*, has published a companion volume entitled *A guide to the trees*,<sup>3</sup> which well sustains the reputation of the former. Nearly two hundred trees are represented, and among them are all those prominent in northeastern America. There is every effort to make determinations easy, by using comparatively few technical terms and by employing the most obvious characters. For example, the

<sup>2</sup> BLANCHAN, NELTJE: *Nature's garden*, an aid to knowledge of our wild flowers and their insect visitors, with colored plates and many other illustrations photographed directly from nature by Henry Troth and A. R. Dugmore. Large 8vo. pp. xvi + 415. New York: Doubleday, Page & Co. 1900. \$3.00.

<sup>3</sup> LOUNSBERRY, ALICE: *A guide to the trees*, with 64 colored and 164 black-and-white plates and 55 diagrams by Mrs. Ellis Rowan, with an introduction by Dr. N. L. Britton. 8vo. pp. xx + 313. New York: Frederick A. Stokes Company. 1900. \$2.50.

primary classification is on the basis of soil, as follows: trees preferring to grow near water (in swamps and by running streams); trees preferring to grow in moist soil (lowlands and meadows); trees preferring to grow in rich soil (forests and thickets); trees preferring to grow in sandy or rocky soil (hillsides and barrens); trees preferring to grow in light or dry soil (upland places, meadows, and roadsides). The arrangement within these five sections is on the basis of leaf characters. The illustrations in color are from originals painted by Mrs. Rowan, and are both artistic and accurate. The pen-and-ink sketches are not so well done, but they are very helpful in determinations.

In the technical description of trees, and in what may be styled the literary appendix to each, the author is on safe ground; but in the pages on "the growth of trees" the statements become ancient in form, vague, and sometimes erroneous. The meaning can be caught by one familiar with the subject, but to the untrained the explanations do not explain.

HARRIET L. KEELER has also written a most attractive book on trees.<sup>4</sup> It is designed to enable the amateur botanist and the general public to recognize trees and so become interested in them. The book is straightforward and matter-of-fact, and is calculated to develop a rational rather than a sentimental or literary interest in trees. The descriptions are clear and simple, and the accompanying remarks in the main have to do with range, time of blooming, general appearance, notable habits, uses, etc. The illustrations from photographs are as perfect as any we have seen, being exceptionally fine examples of photography and half-tone reproduction. Every detail stands out with the distinctness of the original specimens. The same high commendation cannot be given to the drawings and their reproduction, which are in sharp contrast with the exquisite half tones. As in all such books, one finds a venerable and profuse terminology, which has largely outlived its usefulness.

These books can be commended to the general public and to teachers seeking for suggestions of interesting material.—J. M. C.

#### MINOR NOTICES.

FASCICLES 195, 196, and 197 of Engler and Prantl's *Die natürlichen Pflanzenfamilien* have recently appeared. The first contains the completion of the Salviniaceæ and the Marsiliaceæ by R. Sadebeck and the beginning of the Marattiaceæ by G. Bitter. Fascicles 196 and 197, a double number, contain the Sphaeropsidales, Melanconiales, and Hyphomycetes, by G. Lindau.

<sup>4</sup> KEELER, HARRIET L.: Our native trees, and how to identify them; a popular study of their habits and their peculiarities, with 178 illustrations from photographs and 162 illustrations from drawings. 8vo. pp. xxii + 533. New York: Charles Scribner's Sons. 1900. \$2.00.

## NOTES FOR STUDENTS.

IN A SOMEWHAT extended paper on geotropism<sup>5</sup> Dr. F. Noll controverts with much vigor Czapek's theory of geotropism, as expounded recently.<sup>6</sup> Czapek holds that since gravity and centrifugal force act alike upon plants this can only be because both impart mass acceleration, and this can manifest itself only in pressure of tissue elements or even layers of tissue upon one another. Thus there will be minute differences of radial pressure on different sides of the cortex which must be perceived and responded to by the growing cells. Orthotropic organs seek to equalize such pressures on the flanks; plagiotropic organs (dorsiventral or radial) seek to realize a certain difference of pressure to which they are attuned. To support this view he appeals to the elimination of geotropic effects by the clinostat under proper conditions, and to the behavior of dorsiventral organs both flat and rolled or folded. To this theory Noll strenuously objects. He concludes that the clinostat does not eliminate geotropic stimulation, but only converts the one-sided stimulus of gravity into an all-sided intermittent one. In radial organs rotation prevents curvature, but not in dorsiventral organs, which, even on the clinostat, show geotropic (pseudo-epinastic) curvatures.

Experiments on various organs, ingeniously subjected to artificial radial pressure equal to that of gravity, resulted negatively. Furthermore the behavior of twiners is cited to show that gravity acting tangentially produces acceleration of growth as well as when acting radially. Turning to dorsiventral organs, Noll urges that mere rolling or folding does not suffice to produce orthotropism, but that this is a true geotropic response. He cites physiologically dorsiventral but anatomically radial organs, such as the peduncles of *Tropæolum*, *Linaria*, etc., which with age reverse their anatomical structure, to show that the coarser cell structure has nothing to do with geotropic sensitiveness.

A few years ago Czapek discovered that geotropically stimulated roots treated with reducing reagents react differently from unstimulated ones.<sup>7</sup> In his later paper (*l. c.*) he declares that this "makes objectively visible the process of perception." To this Noll objects as going too far; the chemical phenomena are rather secondary and are not closely connected with the perception of the stimulus.

At the close of his paper Noll suggests a possible structure for the receptive apparatus, which must be located in the ectoplasm in order to have the necessary fixed orientation. The plasma has a tendency to form globular centrosphere-like bodies and the receptive apparatus may well be analogous

<sup>5</sup> NOLL, F.: Ueber Geotropismus. *Jahrb. f. wiss. Bot.* 34:457-506. 1900.

<sup>6</sup> CZAPEK, FRIEDRICH: Weitere Beiträge zur Kenntniss der geotropischen Reizbewegungen. *Jahrb. f. wiss. Bot.* 32:175-308. 1898.

<sup>7</sup> Berichte d. deutsch. bot. Gesells. 15:516. 1897.



to the otocyst of some lower animals, and have the form of a centrosphere containing a centrosome of different specific gravity from the liquid in which it lies, the wall being locally sensitive to the pressure of the centrosome and functionally connected with the release of action in the growing zone. Such an organ need not be of visible size, and it is unlikely that it is the already known centrosphere connected with cell division.—C. R. B.

ITEMS OF TAXONOMIC interest are as follows: C. L. SHEAR (Bulletin 23, Division of Agrostology, Dept. of Agric.) has published a revision of the N. Am. species of *Bromus* occurring north of Mexico, recognizing 36 species and 28 varieties, 45 of which are native and 19 introduced, and describing 15 new varieties, 3 new species, and a new subgenus (*Neobromus*).—R. E. SCHUH (Rhodora 2:111-112. *pl.* 18. 1900) has described a new genus of brown algæ, calling it *Rhadinocladia*.—T. F. ALLEN (Bull. Torr. Bot. Club 27:299-304. *pls.* 10-15. 1900) has described three new charas from California.—L. F. HENDERSON (*ibid.* 342-359) has described 26 new plants from Idaho and other northwestern localities.—R. CHODAT (Mém. de l'Herb. Boiss. 17:10. 1900) has described three new genera of Protococcoideæ from the plankton flora of the ponds of Denmark, naming them *Lemmermannia*, *Hofmania*, and *Catena*.—F. STEPHANI (*ibid.* 16:1-46. 1900), in continuing his *Species Hepaticarum*, presents *Calycularia* (6 spp.), *Makinoa* (1 sp.), *Cavicularia* (1 sp.), *Blasia* (1 sp.), *Pellia* (3 spp.), *Androcryphia* (1 sp.), *Petalophyllum* (2 spp.), *Treubia* (2 spp.), *Fossombronina* (40 spp.), *Haplomitrium* (1 sp.), and *Calobryum* (3 spp.).—W. N. SUKSDORF (Deutsch. bot. Monats. 18:86-88. 1900), in continuing the publication of Washington plants, describes new species or varieties in the following genera: *Ribes*, *Epilobium*, *Boisduvalia*, *Godetia*.—JAMES BRITTEN and E. G. BAKER (Jour. Bot. 38:241-246. *pl.* 411. 1900) have published some notes on *Eryngium*, with special reference to certain obscure North American species.—J. W. CONGDON (Erythea 7:183-189. 1900) has published a fascicle of new species from Mariposa co., Cal., representing the genera *Sidalcea*, *Ribes*, *Epilobium*, *Echinocystis*, *Selinum*, *Erigeron*, *Wyethia*, *Cnicus*, *Gilia*, *Collinsia*, *Mimulus*, *Castilleia*, and *Orthocarpus*.—A. ENGLER (Bot. Jahrb. 28:291-384. 1900), in continuing his *Beiträge zur Flora von Afrika*, presents the following contributions: M. GÜRKE on Verbenaceæ (II), Boraginaceæ (I), and Labiatae (V); P. HENNINGS on the fungi of eastern Africa; K. SCHUMANN on a new family of Malvales, which he calls *Triphlochitonaceæ*, the single genus being *Triphlochiton*; A. ENGLER and numerous collaborators on a collection from the lake Nyassa region, abounding in new species.—G. HIERONYMUS (*ibid.* 29:1-35. 1900) has published an account of the Compositae of Ecuador, 270 species being enumerated, many of which are new.—TH. LOESENER (*ibid.* 86-106) has published a second paper on the flora of Central America.—KARL REICHE (*ibid.* 107-119) has published a revision of the South American

family Calyceraceæ.—P. GRAEBNER (*ibid.* 120) has published a revision of *Linnaea*, including *Abelia* under it as a subgenus, the genus thus containing 26 species, 12 of which are new.—The most recent "Contribution from the Gray Herbarium" is by M. L. FERNALD (*Proc. Am. Acad.* 35: 489-573. June 1900). It contains a synopsis of the Mexican and Central American species of the great genus *Salvia*, 209 species being recognized, 57 of which are new; a much needed revision of the Mexican and Central American species of *Solanum* § *Tovaria*, 10 species being presented, four of them new; and some undescribed Mexican seed-plants, 31 in number, chiefly Labiatae and Solanaceæ.—J. M. C.

A NEW THEORY of myrmecophily is proposed by Buscalioni and Huber.<sup>8</sup> Writing from Pará, Brazil, last September, they say that Schimper's attractive theory, that the symbiosis exists on account of the plant's need of protection against leaf cutting ants, not only fails to account for the facts, but is directly contrary to many. They find myrmecophilous plants restricted to regions subject to present or recent inundation, which points to a connection between this condition and the development of myrmecophily. This connection appears simple: when the low regions were overflowed the ants were compelled to take refuge on the trees and shrubs, and naturally sought out the hollow parts in which to stow away their larvae. The fodder provided for the police-like guests is probably to be ascribed to the direct influence of the ants themselves or of the Aphides or Coccidæ which seek it. As the regions remoter from the streams became less subject to overflow the ants may have retained their dwellings, although the need had passed, while the protection to the plants may have given the latter an advantage over competitors.

The authors state certain consequences of their theory, which so far have proved true:

- 1) If a plant genus consisting of some ant-free and some myrmecophilous species has different species in the upland and in the inundated land, as a rule the upland forms will be free of ants, and only those in the inundated region will be myrmecophilous.

- 2) Those myrmecophilous species which occur on dry land may be derived either from those which occur in inundated localities or they are found in localities which were periodically overflowed in earlier times.

- 3) The myrmecophilous plants of deeply inundated regions are mainly trees; those in regions of shallow overflow are shrubs.

The authors promise the publication shortly of thorough investigations on the biology and anatomy of the ant plants.—C. R. B.

IN HIS RESEARCH on rheotropism of roots Juel used a neat device for obtaining a current of water of uniform rate to impinge upon roots under

<sup>8</sup> Beihefte zum Bot. Centralbl. 9: 85-88. 1900.

experiment.<sup>9</sup> To a central disk on the end of a vertical rod are attached six radiating arms of strong brass wire, on which are slipped cork disks, and to them seedlings are fastened in any desired position. This carrier is supported above a circular dish of water into which the roots depend while it is rotated on a clinostat. To prevent the retardation of the water as much as possible three other glass vessels each about 6<sup>cm</sup> less in diameter than the next outer one are cemented to it, thus dividing the water chamber into three concentric spaces 3<sup>mm</sup> wide, in which the rate of movement was practically unaffected by the retarding action of the immersed rootlets.

For rheotropic curvatures roots of *Vicia sativa* (2–3<sup>cm</sup> long) and maize (3–4<sup>cm</sup> long) proved best adapted. Currents varying from 0.8<sup>mm</sup> per second (using a hot air motor) to 0.3<sup>mm</sup> per second were tried. The lower limit of sensitiveness was not reached even at the lowest speed. Curvatures of 15–35° were obtained in 6 hours and 10–65° in 21 hours with currents of 0.8–0.3<sup>mm</sup> per sec., all being positive, *i. e.*, against the current. After the rheotropic curvature has become considerable a counter curvature due to geotropism appears in *Vicia sativa*, producing a sigmoid form.

By means of decapitation and covering the root tip with collodion caps, Juel sought to determine the receptive region. He concludes that it is the growing zone. Whether or not the tip was also sensitive he could not ascertain. Juel is not yet able to decide what factor acts as a rheotropic stimulus, and plans to make further researches.—C. R. B.

W. C. WORSDELL<sup>10</sup> has done most excellent service in bringing together the chief views in the vexed discussion concerning the nature of the ovular structures in Coniferae. There is probably no more difficult bit of morphology in connection with seed-plants, and the scattered literature of the subject needed organization and compact presentation. The problematical structures are the so-called seminiferous scale and the sporangial envelope. To homologize these structures throughout Coniferae seems to be a well-nigh hopeless task, and a definite solution still remains to be reached.

Mr. Worsdell traces the history of the discussion from Linnaeus (1737) to Celakovsky (1897), although its real beginning on the basis of modern morphology may be said to date from the announcement of gymnospermy by Robert Brown in 1827. The author's judgment favors the conclusions of Celakovsky, who sees in the seminiferous scale the first two leaves of an axillary bud, being developed directly in a highly modified form as an outgrowth on the bract. Furthermore, each of these two leaves represents an outer integument of the sporangium, all of the Coniferous megasporangia

<sup>9</sup> JUEL, H. O.: Untersuchungen über den Rheotropismus der Wurzeln. Jahrb. f. wiss. Bot. 34: 507–538. 1900.

<sup>10</sup> The structure of the female "flower" in Coniferae, an historical study. Annals of Botany 14: 39–82. 1900.

having two integuments, the outer being transformed in some cases into the so-called aril. This means that no sporophyll or carpel is present in the group.

This view of the seminiferous scale, which combines the old and separate views that it is an axillary shoot, or a ligular or placental outgrowth, or an outer integument, is certainly ingenious, but has the merit of explaining the well-known varying and transitional abnormalities, the reversed orientation of the bundles, and the diverse structures of such forms as *Abies*, *Cupressus*, *Podocarpus*, *Taxus*, etc.—J. M. C.

A WIDENING KNOWLEDGE of reproduction in the lower forms has shown that the line between sexual and asexual reproduction is not so sharp as was formerly imagined and suggests that even in higher plants where parthenogenesis does not normally occur it might possibly be induced by artificial means. The only clearly proven case of parthenogenesis in spermatophytes is furnished by Juel in his account of *Antennaria alpina*. Shaw's experiments led him to believe that in *Marsilea Drummondii* parthenogenesis may occur under normal conditions.

Shaw's work has been confirmed by A. Nathanson,<sup>12</sup> who finds that in *M. Drummondii* about 90 per cent. of the megaspores produce parthenogenetic embryos. *M. vestita* was then tried, but the isolated megaspores under otherwise normal conditions produced no embryos. Numerous attempts to induce parthenogenesis by using chemicals were unsuccessful, but by raising the temperature parthenogenetic embryos were obtained. In a lot of 750 isolated megaspores at the temperature of the room only one embryo was found; but in a lot of 466 spores, at a temperature of 35° C., 34 parthenogenetic embryos developed. These embryos when placed in moist soil continued to develop exactly like embryos resulting from fertilized egg cells.

In *M. macra* 101 isolated megaspores at the temperature of the room failed to produce a single embryo, but out of 67 megaspores, at a temperature of 35° C., eight developed parthenogenetic embryos.

The writer satisfied himself that the embryos were truly parthenogenetic and not merely adventitious.—CHARLES J. CHAMBERLAIN.

IN THE *Jour. Roy. Hort. Soc.* (24) is printed a paper by H. J. Webber on the work of the United States Department of Agriculture on plant hybridization. The work thus far undertaken has been mainly on oranges, grapes, pineapples, apples, pears, wheat, corn, and cotton. The account is one of progress rather than of practical improvement, and the scientific results are apt to be even more important than the horticultural results. The

<sup>12</sup> Ueber Parthenogenesis bei *Marsilea* und ihre Abhängigkeit von der Temperatur. Ber. d. deutsch. bot. Gesell. 18: 99-110. 1900.

work of the hybridization of oranges and other citrus fruits was done in connection with Mr. W. T. Swingle. The aim has been to secure a hardy orange, one with the loose skin of the mandarin, various changes in quality, and resistance to diseases. In the experiments upon pineapple hybridization the problems presented are to secure better shipping kinds, those with smooth leaves, those resistant to disease, and those with larger fruits and of better quality. The main purpose in the experiments on cotton hybridization has been to improve the upland cotton of the interior by means of the fine Sea Island cotton that at present is grown in such a limited area. In experiments on corn hybridization very few of the important problems have been taken up as yet. Something has been done in the direction of the development of the better yielding races. It is in connection with these experiments upon corn that Mr. Webber has developed a remarkable series of results indicating the immediate effect of pollen, a phenomenon known as xenia.—J. M. C.

A SECOND PAPER has appeared on the fertilization of *Batrachospermum* since my account in 1896. Osterhout<sup>12</sup> agrees with Schmidle that a sperm nucleus passes through the trichogyne into the carpogonium and unites with the female nucleus there, and he figures a stage in the process of fusion. Osterhout also is unable to find a nucleus in the trichogyne. It will be remembered that Schmidle<sup>13</sup> reported that the sperms contained two nuclei, one of which fertilized the carpogonium and the other remained in the sperm or passed into the trichogyne. Osterhout observed only one nucleus in the sperm. If more than one sperm fuses with the trichogyne, their nuclei may enter that structure, but they finally become disorganized. Nuclei that remain in the sperms also break down.

Osterhout's material was fixed after several methods, but that killed in Flemming's strong solution gave the best results. The microtome sections were treated with Flemming's triple stain or with haematoxylin after the method of Heidenhain. The preparations, to judge from the figures, must be much better than any that have yet been made of this plant.—BRADLEY M. DAVIS.

A. C. SEWARD and MISS J. GOWAN<sup>14</sup> have published an extensive paper on *Ginkgo*. An historical account of the development of knowledge concerning this most interesting plant is followed by a detailed description of its structure, and an account of its fossil allies. The authors approve its separation as a distinct group of gymnosperms, and confirm its cycad

<sup>12</sup>OSTERHOUT: Befruchtung bei *Batrachospermum*. *Flora* 87:109. 1900.

<sup>13</sup>SCHMIDLE: Einiger über die Befruchtung, Keimung, und Haarinserction von *Batrachospermum*. *Bot. Zeit.* 57:125. 1899.

<sup>14</sup>The maidenhair tree (*Ginkgo biloba* L.). *Annals of Botany* 14:109-154. pls. 8-10. 1900.

affinities. Its very ancient character is pointed out, the type possibly merging with the Cordaitales in the Palæozoic. During the Mesozoic and Tertiary Ginkgo and its allied forms had a remarkable geographical range, representatives having been discovered in almost all parts of the world.

It is of interest to note that the usual statement that Ginkgo does not occur in the wild state is contradicted, several fine specimens having been found by Mrs. Bishop (Miss Bird) "in the magnificent forests which surround the sources of the Gold river and the smaller Min in western China."—J. M. C.

THE ANATOMY of galls has been recently investigated by Küster.<sup>25a</sup> The paper treats also of the development and morphology of these peculiar structures. Great attention is paid to galls produced by insects, but those which are due to myxomycetes, fungi, algæ, or worms are considered incidentally.

The principal results are the following: the simplest structure is found where the growth of the infected part is superficial, but widespread histological changes occur when there is growth in thickness. Galls which arise through the enlargement of cells already present are very primitive in their anatomical structure.

The epidermis withstands for the longest time the influence of the irritation, the mesophyll, cortex, and pith being more easily affected. The upper side of the leaf seems less liable to change than the lower. The most important change in the epidermis is the formation of hairs. Stomata often remain permanently open, and in some cases genuine lenticels are formed underneath them.

Assimilative tissue is scanty, but mechanical tissue is usually formed when there is any growth in thickness. The vascular system is feebly developed. The larva-chambers are surrounded by mechanical tissue and in shape repeat the form of the galls. In general, the cells of galls have the same form and arrangement as in normal tissues, but forms of cells and tissues appear in the galls which are not found in normal parts of the same plant, though found in nearly related plants. However, many galls show cell and tissue forms which are not to be found in related plants.

Experimental plant physiology has not yet succeeded in producing new organs or new cell formations. Galls furnish the only evidence that a plant through artificial external influences can produce new tissue forms or cell forms.—CHAS. J. CHAMBERLAIN.

DR. R. H. TRUE, continuing his studies upon the toxicity of acids and salts, finds<sup>25b</sup> that, given the degree of ionization of an acid and its sodium

<sup>25a</sup> KÜSTER, ERNST: Beiträge zur Kenntniss der Gallen-anatomie. Flora 87: 117-193. 1900.

<sup>25b</sup> TRUE, R. H.: The toxic action of a series of acids and of their sodium salts on *Lupinus albus*. Am. Jour. Sci. IV. 9: 183-192. 1900.

salt, the toxic action may be analyzed into the effect of the H ions, the anions, and the undissociated molecules if any. The latter play a particularly important part, especially in the fatty and aromatic series of acids. In general the H ions of inorganic acids are powerfully toxic; the anions of organic acids are slightly toxic, often negligibly so as compared with the H ions; and carboxyl H is many times more toxic than hydroxyl H.—C. R. B.

MR. F. H. KNOWLTON has brought together our knowledge of the fossil plants associated with the lavas of the Cascade range, and has published the result in the Twentieth Annual Report (Part III) of the United States Geological Survey, in connection with an account of the Bohemia mining region of western Oregon. The species number about thirty, including but three ferns and three gymnosperms, and are said to point unmistakably to the Miocene age of the beds.—J. M. C.

PROFESSOR W. A. KELLERMAN, of Ohio State University, and his wife have published an account of the non-indigenous flora of Ohio (University Bulletin, Botanical Series no. 4). In a state known to contain 2025 seed-plants, it seems that 430 of them are not indigenous. These introduced forms are from the following sources: 326 from Europe, 30 from Asia, 2 from Africa, 46 from South and West United States, 21 from Tropical or South America.

PROFESSOR K. MIYAKE, of the imperial University, Tokyo, finds that starch is present in the leaves of evergreens in winter, and that it is due to feeble photosynthesis occurring during that season. The mean temperatures of various days when this process was determined varied from 0.7–7° C. (mostly less than 3°)<sup>16</sup>.—C. R. B.

DR. G. N. BEST has revised the North American species of *Pseudoleskea*.<sup>17</sup> He recognizes seven species, with four varieties, of which three are new. One species, *P. falcicuspis* Kindb., is excluded; and one, *P. atricha* Kindb., is doubtful.—C. R. B.

<sup>16</sup> Bot. Mag., Tokyo, 14: 44. 1900.

<sup>17</sup> Bull. Torr. Bot. Club 27: 221–236. pl. 6, 7. 1900.

## NEWS.

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DR. ROLAND THAXTER sailed for Europe late in June for about two months' absence.

DR. B. M. DAVIS is in charge of the botanical department of the Marine Biological Laboratory at Woods Hole during the summer.

THE *Fern Bulletin* for July publishes a sketch of Daniel Cady Eaton by Professor Setchell, accompanied by an excellent portrait.

DR. B. L. ROBINSON is now in Europe, where he will spend much of the summer examining types of American species in European herbaria.

IN *Rhodora* for June there appears a sketch of Edwin Faxon by Mr. George G. Kennedy, accompanied by an unusually well-executed portrait.

MR. A. C. MOORE, a fellow in botany at the University of Chicago, has been appointed professor of biology in South Carolina College, Columbia, S. C., the state institution.

DR. P. B. KENNEDY, of the Division of Agrostology, U. S. Department of Agriculture, has been appointed associate professor of botany and horticulture in the University of Nevada.

MR. GEORGE P. CLINTON, instructor in botany, University of Illinois, has a leave of absence for the year 1900-1, to study at Harvard. His principal study will be the Ustilagineæ and their allies.

DR. WALTER BUSSE, docent in the University of Berlin, has undertaken a journey through the steppes of German East Africa in search of technically and medicinally valuable plants and plant products.

PROFESSOR GEORGE L. GOODALE sailed in May for Europe. It is his sabbatical year, and he will be away during the coming college year. His work will be done by Dr. R. H. True and Mr. E. W. Olive.

MR. M. L. FERNALD will spend two weeks of July in a botanical exploration of Mt. Katahdin, Me., with J. F. Collins, of Brown University, Judge J. R. Churchill, Dr. G. G. Kennedy, and Émile F. Williams, of Boston.

DR. H. C. COWLES conducted a class in field study of ecology through the Tennessee mountains during June. He has been appointed to take charge of the botanical work at the Biological Laboratory at Cold Spring Harbor during the summer.



MR. C. F. HOTTES, assistant in the botanical department of the University of Illinois, went two years ago to Bonn to equip himself for taking up as a specialty vegetable physiology. His work has proved so satisfactory that he intends staying another year. He will return to the University in September 1901.

A PROSPECTUS of an illustrated work on the maples, native and cultivated, in North America, has been issued by A. M. Lochman, Bethlehem, Pa. The work is to be by Charles L. Lochman, and is to have twenty-four full-page half-tone plates made from photographs of shoots and drawings, with forty-eight pages of text.

PROFESSOR DR. E. LOEW (Berlin, S. W., Grossbeerenstr. 67) and Dr. Otto Appel (Charlottenburg, Schloss-str. 53), who have undertaken to prepare the third volume of Knuth's *Handbuch der Bluthenbiologie*, appeal to all who have made biological observations on extra-European plants to communicate their papers or notes.

MR. B. T. GALLOWAY, chief of the division of vegetable physiology and pathology in the U. S. department of agriculture, was elected a vice-president of the A. A. A. S. and chairman of Section G (botany) for the Denver meeting. Professor A. S. Hitchcock, of the Kansas Agricultural College, was elected secretary of the section.

BACTERIOLOGICAL EXAMINATIONS of the Chicago drainage waters are constantly in progress in the University of Chicago and the University of Illinois. Samples from a score of places along the sanitary canal and the Illinois and Mississippi rivers are examined each week with a view of determining the extent and kind of sewage contamination, and the influence of natural conditions in the purification of the stream.

IT IS EXPECTED that there will be no changes at present in the staff at the Missouri Botanical Garden and the Shaw School of Botany. During the summer Dr. H. von Schrenk will make a study of the diseases of forest trees, in the interest of the United States Department of Agriculture, probably spending most of his time in the Pacific coast region. Mr. H. F. Roberts will pass the summer at the Marine Biological Laboratory at Woods Hole.

THE SECOND ANNUAL SESSION of the Rhode Island Summer School for nature study was held at the Rhode Island College of Agriculture and Mechanic Arts, Kingston, R. I., July 5th to 20th, 1900. In addition to Professor H. L. Merrow of the regular staff of the College and Experiment Station, the following botanists took part: Professor W. W. Bailey and Mr. J. F. Collins, Brown University; Professor J. M. Macfarlane, University of Pennsylvania; and Dr. A. B. Seymour, Harvard University.

THE FOLLOWING NOTE, written in response to our circular letter requesting for review a copy of the book named, may be of interest to botanists who

read reviews of it. They will at least know the price which the publishers offer for "a good review" and the "terms" on which, presumably, the book is advertised by such "scientific magazines" as receive copies:

DEAR SIR: Referring to your memorandum of July 23d, no copies of McIlvaine's *One Thousand American Fungi* have been or will be sent out for review. We have sent a few copies to the editors of scientific magazines, allowing them a special discount of 50 per cent. off the list price in consideration of a good review. Should you care to review the book on these terms we will be glad to send you a copy for \$6.

Very truly yours,

THE BOWEN-MERRILL CO.

ON AUGUST FIRST Dr. C. E. Bessey returned to active work in the botanical department of the University of Nebraska. He celebrated his resumption of botanical work by a visit to the Yellowstone Park for the purpose of studying the vegetation of that region, especially certain groups of lower plants. With his return to the department Dr. August Rimbach's connection will shortly cease, as he was engaged in the department during Dr. Bessey's year of service as chancellor. Professor John L. Sheldon, of the Nebraska State Normal School, has accepted a fellowship in botany in the university, and will return to study at the opening of the year. Dr. F. E. Clements, at the close of the Summer School, goes to the Pike's peak region again for further ecological studies of the alpine and sub-alpine vegetation, continuing also his collection of specimens for distribution.

THE PRIVATE HERBARIUM of Mr. Harry N. Patterson, of Oquawka, Ill., containing about 30,000 sheets, has been secured by the Field Columbian Museum, and will be installed with the rapidly growing collections of that institution as promptly as the careful cataloguing practiced in all departments will admit. The botanical department of the museum is to be congratulated upon this accession of one of the notable private herbaria of the country; one that will add a complete collection of Pringle's Mexican plants to its already excellent representation of the flora of that region and the Antillean islands. Mr. Patterson's herbarium is more or less contemporaneous with that of the late Mr. Bebb, which the museum secured some three years ago, and as Mr. Patterson made it his aim to secure a complete series of the species of North America, its addition to the collections of the museum will be of great value to botanical students and specialists in the west.

# BOTANICAL GAZETTE

SEPTEMBER, 1900

## ORIGIN OF THE CONES OF THE MULTIPOLAR SPINDLE IN GLADIOLUS.

ANSTRUTHER A. LAWSON.

(WITH PLATE XII)

RECENT investigation in cytology has gone far to establish the fact that multipolar spindles are of very general occurrence in the higher plants. It is indeed held by many investigators that the origin and development of the karyokinetic spindle in these plants is diametrically opposed to that which prevails in the lower plants and animals. These observers believe that the spindle, instead of being bipolar from the first and under the control of a kinetic center, passes through a series of multipolar stages in the course of its development. The problem has thus become exceedingly interesting and important, and calls for vigorous and careful investigation.

Multipolar spindles have been found in *Lilium* by Farmer (1893), in *Larix* by Belajeff (1894) and Strasburger (1896), in *Equisetum* by Osterhout (1897), in *Lilium*, *Fritillaria*, *Helleborus*, *Podophyllum*, and *Pinus* by Mottier (1897, *a* and *b*), in *Hemerocallis* by Juel (1897), in *Chara* by Debski (1897), in *Zamia* by Webber (1898), in *Sagittaria* by Schaffner (1898), in *Nymphaea* and *Nuphar* by Guignard (1898), in *Hesperaloe*, *Hedera*, *Disporum*, *Smilacina*, *Gladiolus*, *Iris*, and *Cobaea* by Lawson (1898), in *Passiflora* by Williams (1899), in *Solanum* by Nemec (1899), and in *Convallaria* and *Potamogeton* by Wiegand (1899).

Occurring in such a wide range of forms, and having been demonstrated by various methods of fixing and staining, it seems extremely improbable that the phenomenon is abnormal or due to artifact. Such a conclusion is much strengthened by the fact that in none of the higher plants has the animal type of spindle formation been discovered. While it is true that some investigators (Guignard, Schaffner, Fullmer, etc.) have described and figured granules situated at the poles of the spindle, and thus in some respects resembling centrosomes, in no case have such granules been observed to take an actual part in the formation of the achromatic figure as it occurs in animals.

Assuming then that the multipolar type is the normal type of spindle formation in the higher plants, since Strasburger and other authorities cannot reconcile the function of a kinetic center with such a process, we are confronted with two problems, namely, the manner in which the multipolar spindle becomes bipolar, and the origin of the cones.

No explanation has yet been offered that will reasonably account for the ultimate bipolarity of the spindle beyond the mere statement that certain of the cones fuse and arrange themselves in two groups. Such an explanation was first suggested by Belajeff (1894), and was later confirmed by Osterhout (1897), Mottier (1897), and others. How this fusion is brought about is still uncertain. Even Guignard (1898), who describes centrosomes situated at the apices of the cones in *Nymphaea* and *Nuphar*, states that he is "unable to suggest, at present, in what manner the multipolar spindle becomes bipolar." So that with or without centrosomes the phenomenon is unaccounted for, and we must therefore wait for the light of future investigation.

On the origin and development of the cones much more encouraging results have been obtained. The investigations of Belajeff (1894) and Strasburger (1896) on *Larix*, Osterhout (1897) on *Equisetum*, Mottier (1897) on *Lilium*, Juel (1897) on *Hemerocallis*, and more recently those of Nemec on *Solanum* and *Allium*, and Davis on *Anthoceros*, have thrown considerable

light on the subject. But while these observers all agree that the spindle arises from a weft of kinoplasmic threads, very few observations have been made on the very earliest stages of the development of the cones; probably on account of the fact that these early stages are very difficult to obtain. In 1898, however, the writer was fortunate enough to obtain a very complete series of these early stages in the pollen-mother cells of *Cobaea scandens*. In this case it was found that, as division approaches, the nucleus becomes surrounded by a zone of granular substance which differs in structure and staining properties from the rest of the cytoplasm. This zone is so constant that it was proposed to designate it perikaryoplasm, and as it has been found in several other genera, the writer feels justified in using the term in the present paper. Upon the breaking down of the nuclear wall, the perikaryoplasm forms a central network of kinoplasmic fibers which grows out into several projections. By their growth outward these projections become the cones of the multipolar figure. The spindle fibers are therefore formed by the elongation of the meshes of the net-work in the direction of the projections.

Encouraged by these results the writer was led to pursue his investigation of these early stages in other forms. His observations on *Gladiolus* proved so interesting that it was thought advisable to record them, believing that they will throw further light on this much disputed problem.

The method employed in preparing the material was practically the same as that adopted in my work on *Cobaea*, namely: Flemming's strong solution of chromic-osmic-acetic acid, diluted with one volume of water, was used for fixing, and the triple stain, safranin, gentian-violet and orange G, for staining. I would here like to emphasize the importance of fixing the material in the field, because of all the material I have examined it was only in those anthers which were fixed *immediately* after being detached from the plant that the early multipolar stages were found. After being fixed the anthers were thoroughly washed in running water from six to eight hours, and were then

dehydrated by being passed through various grades of alcohol. Bergamot oil was used to precede the infiltration of paraffin. Microtome sections from  $3.6\ \mu$  to  $6\ \mu$  thick were used.

THE POLLEN MOTHER CELLS OF GLADIOLUS.<sup>1</sup>

Gladiolus affords very exceptional material for the study of spindle formation on account of the large size of its anthers, pollen mother cells, and nuclei. The cytoplasm in the resting pollen mother cell appears in the form of a clear uniform reticulum, with numerous small spherical bodies scattered irregularly through it. The nucleus is very large, containing a vacuolated nucleolus, and the chromatin in a very characteristic spirem. As division approaches, the cytoplasm undergoes a remarkable differentiation in identically the same manner that occurs at this stage in the corresponding mother cells of *Cobaea*. While the chromosomes are being formed, there gradually accumulates a complete and sharply differentiated zone of granular substance about the nucleus. In nearly every respect this zone resembles the perikaryoplasm so characteristic of *Cobaea*. It accumulates in the same manner, has the same structure, and stains in the same fashion. In fact, the only difference that could be detected was in the size of the granules of which the zone is composed. In *Gladiolus* these granules are very fine, while those in *Cobaea* are comparatively coarse. We shall therefore continue to use the term perikaryoplasm in the following description.

By the time the chromatin thread breaks up and assumes the form of curved rod-shaped chromosomes, the perikaryoplasm has reached its maximum development. In many cases it was observed that the small black spherical bodies which were scattered irregularly through the cytoplasm had arranged themselves in the form of a ring at the outer margin of the perikaryoplasm. But this peculiarity is not as constant or as striking as it is in *Cobaea*.

At this early stage in *Cobaea* it was observed that the nuclear membrane breaks down, but in *Gladiolus* this does not happen

<sup>1</sup> One of the common cultivated garden forms, probably of the hybrid *G. Gandavensis* (Hort.).

until a much later stage, as can be seen readily in *figs. 1-8*. This fact is significant, because it is quite clear that the method of spindle development depends much upon *when* the nuclear membrane breaks down. This is very well illustrated by the two types which we have before us. In *Cobaea*, where the nuclear wall breaks down at a very early stage, we have a central network formed which occupies the space of the nuclear cavity. In *Gladiolus*, where the nuclear wall persists, the network is not formed in this central position, but is formed outside of and immediately surrounding the membrane. This difference also has its effect on the character of the network. When the nuclear wall breaks down at an early stage the nuclear cavity affords a large space for the formation of the network. Its meshes are consequently quite large. On the other hand, when the nuclear wall persists, as it does in *Gladiolus*, the network appears in the form of a close weft or felted zone.

Very careful observations were made at this stage to see if any of the kinoplasmic threads penetrate the nuclear membrane, as recently observed by Wiegand in *Potamogeton*. Although hundreds of cells were examined no such penetration was detected.

When this weft or felted zone commences to form, one might at first think that it arose by the fraying out of the nuclear membrane. But as it grows to such a width (*fig. 1*), and the nuclear membrane apparently loses none of its distinctness (*figs. 1-8*), it seems much more probable that it grows at the expense of perikaryoplasm as the central network does in *Cobaea*.

From the time of its first appearance the felted zone stains very readily with gentian-violet, and as it increases in size its meshes become larger and the sharp kinoplasmic threads of which it is composed are readily distinguished. As soon as the weft reaches a certain size (*fig. 1*), it ceases to grow uniformly, but pushes out into several projections as shown in *fig. 2*. As far as the writer could make out, there appears to be no definite number of these projections. *Fig. 2* shows at least five. Six was the largest number observed in cross-section. Just how

many there are it is impossible to say at present. These projections rapidly increase, and on account of their growth outward the meshes become decidedly elongated. By the time they have reached their full growth, the whole outer portions of the cones are composed of long distinct fibers converging to the apices, and it is only at the base that the meshes of the original weft can be distinguished. The apices of the cones taper out into remarkably sharp points, but in no case was there a body observed at these points which might be considered as a controlling center.

During the entire process of the formation of the cones the nuclear membrane remains intact. It is only after they have reached their full development that it begins to break down. The breaking down of the membrane is shown in *figs. 6 and 7*. It will be seen from these figures that this takes place on one side first, where the identity of the membrane becomes lost in the network at the base of the cones. It will also be observed from these figures that the nucleolus still persists. It remains quite conspicuous until all traces of the nuclear wall are lost, when it suddenly disappears. What eventually becomes of it was not observed.

In my observations on *Cobaea* it was thought probable that the linin of the nucleus took part in the formation of the central network from which the cones develop. Now in *Gladiolus*, where the kinoplasmic network and the cones are fully developed before the nuclear wall disappears, it becomes quite evident that the linin takes no essential part in the formation of the achromatic figure.

Upon the disappearance of the nuclear membrane the bases of the cones soon adjust themselves to the space offered by the nuclear cavity, and in doing so come in direct contact, for the first time, with the chromosomes. These latter bodies are not lacking in interest; but as the writer, in future work, hopes to make a more detailed study of the chromatin in *Gladiolus* and other forms, his observations will not be recorded at present. Soon after this stage a series was observed showing that the



cones first approach each other and finally unite in two groups in the form of a bipolar spindle, as shown in *fig. 10*.

From the above observations, and from previous work on other forms, it would seem that there are several types of spindle development in the higher plants. It would be imprudent to classify all of these probable types until the early stages of many of the forms have been more thoroughly investigated. At present we have at least three forms which have been thoroughly worked out and which differ from one another sufficiently to warrant us in distinguishing them as types. These are represented by *Equisetum*, *Cobaea*, and *Gladiolus*.

#### SUMMARY.

The above observations may be summarized as follows :

As nuclear division approaches, a granular zone accumulates about the nucleus. This zone in every respect resembles the perikaryoplasm so characteristic of the pollen mother cells of *Cobaea*.

A close network or felted zone of kinoplasmic fibers is formed immediately outside of and completely surrounding the nuclear wall. This is probably developed from the perikaryoplasm.

This network grows out into several projections which become the cones of the multipolar figure.

The nuclear membrane persists until the cones are almost fully developed.

The spindle fibers are formed by the elongation of the meshes of the network composing the cones.

Neither the nuclear wall, nucleolus, nor linin take any essential part in the formation of the achromatic figure.

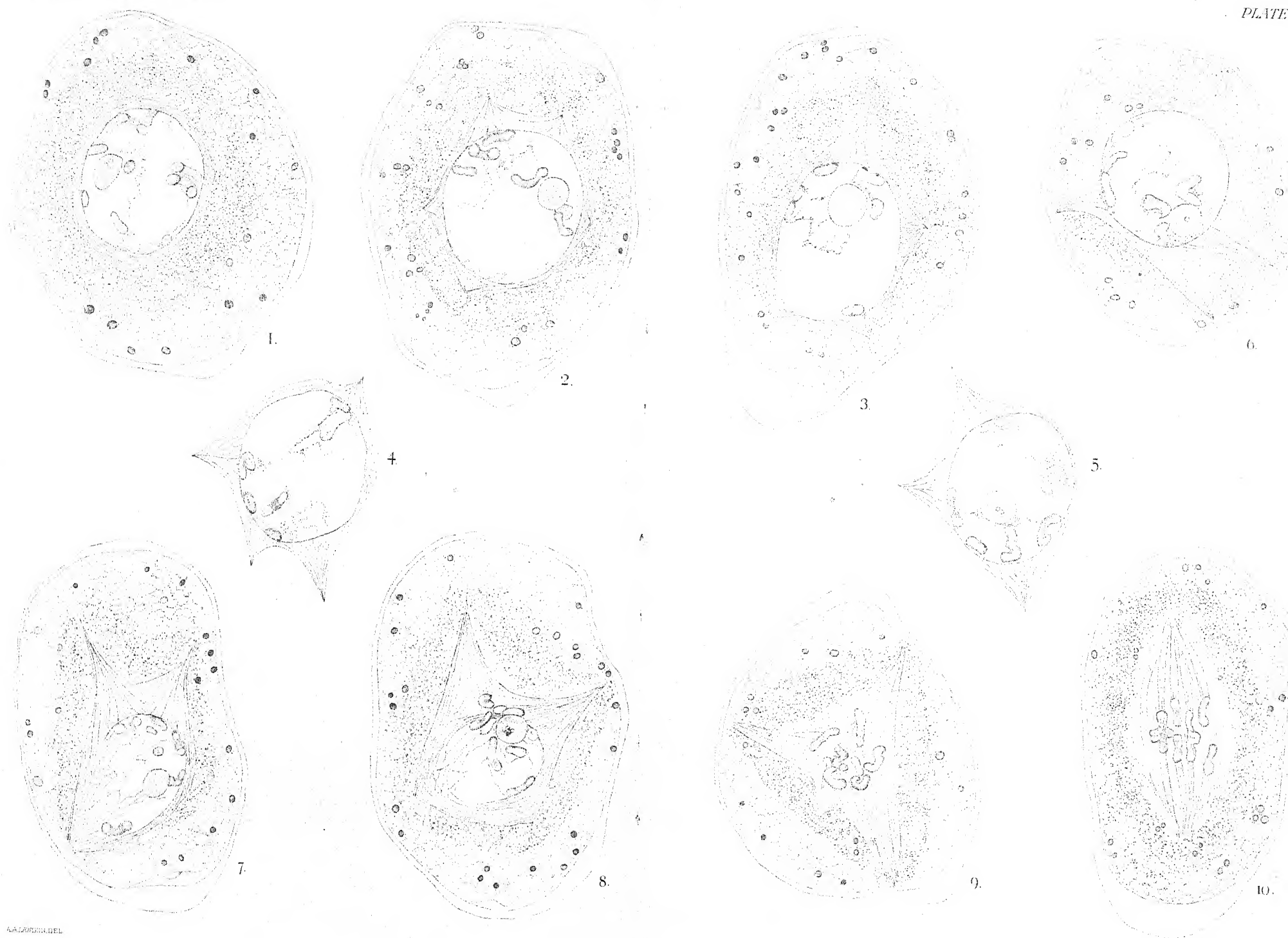
The cones of the multipolar figure fuse and arrange themselves in two groups and form a bipolar spindle.

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ALANON DEL

LAWSON on GLADIOLUS

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## EXPLANATION OF PLATE XII.

Figures drawn with Abbe's camera lucida, Zeiss homog. immersion objective one twelfth, apert. 1.25, compensating ocular no. 6.

FIG. 1. A pollen mother cell showing the cytoplasm differentiated into three zones; the outer cytoplasm stains a light gray-blue; the perikaryoplasm stains a light orange, and the weft or felted zone of kinoplasmic fibers stains blue; the nuclear wall is intact and the nucleus contains a large nucleolus and several curved chromosomes, with a small amount of linin threads.

FIG. 2. The weft of kinoplasmic threads is commencing to push out into several projections, preparatory to forming the cones of the multipolar figure; the network of the weft appears more distinctly.

FIG. 3. A still later stage in the development of the projection, with distinct cones formed.

FIG. 4. A still later stage showing the elongation of the meshes and the formation of fibers by the pulling out of the network.

FIG. 5. A slightly older stage than that of *fig. 4*.

FIG. 6. A later stage when the cones are nearly fully developed; the outer portions of the cones consist now of distinct fibers, and it is only at the base of the cones that the network of the original weft can be distinguished; the nuclear wall has as yet shown no sign of breaking down, and the nucleolus still persists.

FIG. 7. The first indication of the nuclear wall breaking down. The chromosomes now come in contact with the base of the cones.

FIG. 8. Shows the same as *fig. 7*, but a little more advanced.

FIG. 9. The nuclear wall and the nucleolus have disappeared; several of the cones have fused, and the chromosomes are attached to the fibers at the base of the cones.

FIG. 10. The mature spindle, with the chromosomes at the equator and about to move to the poles.

## THE DEVELOPMENT AND FUNCTION OF THE CELL PLATE IN HIGHER PLANTS.

H. G. TIMBERLAKE.

(WITH PLATES VIII AND IX)

(Concluded from p. 99)

### 2. *The genetic stage.*

THE origin of the cell plate elements occurs in the equator of the central spindle. In the onion this brings them in the midst of the carbohydrate zone (*figs. 18, 19*). There seems to be no doubt that they are thickenings of the spindle fibers. In the onion, while they are often very difficult to distinguish at first, owing to the fineness of the fibers and the abundance of carbohydrate material, it can be determined clearly that they are swellings of the fibers (*fig. 29*). There is nothing to suggest the movement of cytoplasmic granules toward the equatorial plane to form the cell plate in the manner described by Treub. Working on living cells, Treub might easily have failed to see the beginning of the cell plate. His statement that it first appears as a fine line would indicate this. The same explanation would apply to the observations of Zacharias, although it is to be noted that Zacharias described special bodies as cell plate elements and not mere undifferentiated cytoplasmic granules such as those of Treub. It is to be doubted, however, whether these bodies have any connection with a cell plate. I do not think it unreasonable to suppose that what Zacharias saw was a substance destined for the formation of the cell wall instead of a cell plate. He may possibly have seen the hyaline spaces between the spindle fibers which I have found stained orange, as above described.<sup>36</sup> The origin of the cell plate elements is shown more clearly in the larch. Here the thickenings are much more pronounced, being elongated bodies instead of small

<sup>36</sup> Cf. FARMER, p. 78 of this paper.

nodules (*figs. 4, 27c*). In this case it looks as if the activity of a fiber previously described as beginning near either daughter nucleus and going toward the equator, has become localized at the latter point, producing a swelling on the fiber. The production of such a swelling is accompanied by the further shortening of the fiber (*fig. 27c*), showing that there has been an actual transformation of the substance of the fiber in the formation of the cell plate element. While the above described process seems to agree with the older observations of Strasburger that the substance of the cell plate elements has flowed in the fibers to the equator,<sup>37</sup> it should be noted that there is nothing in the process I have described to indicate a collection of smaller granules within the fiber, but that the fiber itself has changed. The whole process seems to indicate a somewhat plastic character of the fiber.

The time relative to other phases of mitosis at which the cell plate elements occur seems to vary in the onion and the larch. In the former their appearance is concurrent with or closely following that of the carbohydrate material. The chromosomes have begun at this time to form slightly denser masses, preparatory to the reconstruction of the daughter nuclei, but separate chromosomes may still be distinguished. In the larch, the process of reconstruction goes so far before the cell plate elements appear that the daughter nuclei are often clearly outlined (*figs. 3, 4*). Whether this difference has any special significance, I cannot say. It would be interesting to compare a wider variety of forms in this respect.

As soon as the elements can be detected, they seem to form a layer throughout the thickness of the original spindle, indicating that they are simultaneous in their origin. Very early stages may have escaped observation, however, although a careful search was made for them. That such a progressive formation might be the case would be suggested by such figures as 5 and 6, where there are some peripheral fibers on which cell plate elements have not yet formed. On the other hand, it

<sup>37</sup> Cf. p. 75 of this paper.

is probable that all the fibers showing cell plate elements in *fig. 4* are the original connecting fibers, while the peripheral fibers are the previously described radiating fibers. I have previously shown that the changes in the connecting fibers, before the formation of the cell plate elements take place in all of the fibers at the same time, while the radiating fibers have apparently so changed as to form peripheral connecting fibers. Whether all of the connecting fibers form cell plate elements was a point that I could not settle with certainty. It is possible that those connecting fibers which did not show the changes described in the preliminary stages do not form cell plate elements. The phenomena observed in later stages render such a possibility more probable.

After they first become visible, the cell plate elements continue to enlarge in their equatorial diameter until they come into contact with one another and fuse into a continuous plate. This process is accompanied by the further shortening of the fibers. In connection with the shortening of the spindle fibers there is a continued appearance of trophoplasm in the terminal parts of the spindle (*figs. 4, 5*).

The result of the processes just described may be briefly summed up as follows. There is in the spindle a young cell plate formed simultaneously from the substance of the spindle fibers. In the onion it lies in the midst of a zone of reserve carbohydrate material to be used in the formation of the new cellulose wall. In the larch such a zone is not seen at this stage. The cell plate may now be said to begin the next stage in its development.

### 3. *The growing stage.*

This stage is marked by the following phenomena: The central fibers continue to shorten, adding their substance to the cell plate until they have finally disappeared. The peripheral fibers begin to appear more and more bent and to form cell plate elements. As the central fibers disappear their place is partly taken by granular trophoplasm and the daughter nuclei come to lie nearer the young cell plate. While the peripheral



growth is continuing, the older portion of the cell plate splits, and the new wall is laid down between the halves. These processes continue until division is complete. During the period of growth the cell plate may so shift its position as to lie in a plane different from that in which it was first formed.

I have thought it best to describe the above processes separately. The fate of the spindle fibers is a question of much interest here. From the phenomena that I have been able to observe in the cells studied, I have become convinced that all of the fibers that form cell plate elements are completely used up in the growth of the cell plate. A comparison of *figs. 4, 5, 6, and 7* is instructive on this point. In *fig. 4* the cell plate elements have just formed, the connecting fibers have drawn away from the daughter nuclei, and granular trophoplasm has appeared among the ends of the fibers of the central spindle. In *fig. 5* the cell plate elements have fused into a cell plate, the fibers have shortened still further, and the trophoplasm appears as before. *Fig. 6* is especially important. Here the fibers have become very short and the ends furthest away from the cell plates are no longer surrounded by the granular trophoplasm. These ends appear to be sharp pointed, while the portion of each fiber that is in connection with the cell plate is relatively thick. At a later stage the trophoplasm becomes distributed more throughout the region originally occupied by the central spindle. Here it frequently appears in irregular rows of granules (*fig. 7*). In this latter figure very small portions of the spindle fibers may be seen. They show the typical pointed structure above described and are distinct from the rows of trophoplasm. Throughout all of the processes involved in the formation of the cell plate the spindle fibers and the cell plate itself stain strongly with the violet of the triple stain. I have not observed the gradual transition from the violet to the orange color described by Strasburger<sup>38</sup> for the spindle fibers at the time when the cell plate is formed. Not only do the spindle fibers and the cell plate stain violet, but the plasma membrane of the

<sup>38</sup> Zellhäute.

mother cell frequently shows the same color. To be sure some preparations show the orange color in connection with the cell plate, but the violet of the cell plate itself could nearly always be distinguished. The orange color is probably due to the small amount of carbohydrate material that sometimes appears at this stage. I do not think that the presence of the above mentioned irregular rows of granular trophoplasm is to be taken as evidence that the spindle fibers break up into granular cytoplasm. My preparations show clearly that the spindle fibers are entirely distinct from these rows. The history of the fibers seems to consist of a gradual shortening and thickening until their whole substance is transformed into a cell plate, *i. e.*, a membrane. While the above description will apply to those fibers which take part in the formation of the cell plate, the fate of those radiating fibers which have no part in this process remains unaccounted for. My observations upon this latter point have been too limited to base any conclusions upon them. In a very early stage in the formation of the cell plate these fibers often seem to lose their characteristic radial arrangement, and to become more of a tangled mass in the cytoplasm (*figs. 3, 4*). It is possible that they become separated from the daughter nuclei and are finally absorbed into the rest of the protoplasm. There is one other interesting case to be noted. In some cells of the larch and some of the cells of the root tip of *Fritillaria*, after the cell plate was formed, a few clearly defined fibers were observed in the region between each daughter nucleus and the cell plate. These fibers are probably the same that have been mentioned previously as not showing the stages preparatory to the formation of the cell plate. Their fate was not determined. *Fig. 10* is interesting in this connection. It represents a late stage in the division of the pollen mother cell when the permanent cell plates are complete, but when there still exist numerous fibers all around each nucleus, extending into the cytoplasm and reaching in many cases to the plasma membrane. Whether these are a part of the connecting and radiating fibers that existed during the earlier stages, or new

fibers that have been formed after the division was complete, I did not determine. If the former supposition is true there would seem to be an unusually large number of spindle fibers which took no part in the formation of the cell plate. From the fact that in all cases observed of the formation of the first cell plate no such abundance of regularly arranged fibers was evident, I think it probable that these are mostly new fibers. Connecting stages, however, may have been overlooked. Their significance in either case is not clear. It may be that they build a new plasma membrane around each pollen grain inside of the plasma membrane of the special mother cell. Further investigation upon these late stages of the formation of the pollen grains is desirable.

The process by which the cell plate grows in area is difficult to determine with certainty. By comparing stages represented in *figs. 4-7*, I have concluded that a part of the growth of the cell plate takes place by means of the continued addition to it of the substance of the original fibers until they are entirely used up. I have already described what seems to be the process through which the fibers go in the formation of the cell plate. I consider the evidence for the complete transformation of at least part of the fibers into the cell plate conclusive. The most reasonable interpretation of *fig. 6* seems to me to be that all of the very short fibers here shown are the remains of the connecting spindle fibers shown in *fig. 4*. This interpretation is further strengthened by an examination of an intermediate stage (*fig. 5*). Here the fibers are not much shorter relatively than they are in *fig. 4*, but it is noteworthy that they are further apart, while the cell plate is, of course, more conspicuous. These facts point to the conclusion that the substance of the fibers, collecting in the middle, has simply pushed the fibers further apart, a process that constitutes the growth of the cell plate. While such a simple mechanical process may account for a portion of the growth of the cell plate, there are phenomena which seem to indicate that other processes accompany it. In all of the above figures, while there is a large area throughout in

which the cell plate is in the same stage of development, it will be noted that the peripheral portions show earlier stages, and that there are fibers connected with this latter portion whose substance has not yet been used in the formation of the cell plate. In many cells these fibers show all gradations from very short ones toward the center of the cell to those reaching nearly, if not quite, to the daughter nuclei (*fig. 30*). Some of the latter, instead of extending from one nucleus to the other, extend simply from the nucleus into the cytoplasm of the opposite daughter cell. These facts would indicate that these peripheral fibers are radiating fibers which are taking part in the formation of the cell plate. The fact that the peripheral fibers appear to be more numerous and in a more compact layer in the stages represented by *figs. 6* and *7* than in earlier stages (*fig. 5*) may be explained by the crowding produced by the growth of the cell plate by the transformation of the inner fibers in the fashion described above, *i. e.*, the peripheral fibers in *fig. 6* would represent all of the radiating fibers between the boundary of the cell plate in this figure and its boundary when it had reached a stage such as that shown in *fig. 5*. The appearance of shorter fibers on the inside of the peripheral bundles and longer ones on the outside shows that the process of cell plate formation goes on gradually in these fibers from the inside outward. This is shown farther by the fact that the cell plate elements of the inner fibers have already fused into a cell plate, while the intermediate ones are often distinct, and the outer fibers often show no cell plate elements (*fig. 30*). The foregoing observations seem to show that the increase in area of the cell plate is due to a two-fold process consisting (1) of a continuous transformation of the substance of the original connecting fibers, resulting in the expansion of the portion of the plate around each fiber and the consequent wider separation of the fibers; (2) concurrent with the above method occurs the addition of new cell plate elements to the peripheral portions of the cell plate, and their growth in the same fashion as those previously formed. These latter processes go on in the radiating fibers which have come into

such a relation to the central spindle as to continue the first process of growth.

Another question of importance in this connection is, does the growth in area of the cell plate depend entirely upon the fibers already existing, or are new peripheral fibers formed? So far as my observations upon the larch go, I think it probable that there is no formation of new fibers, but that the whole growth takes place as a result of the changes in the existing fibers; yet the evidence on this point was not conclusive. If the hypothesis is true, whether the mother cell divides into the four cells which form the pollen grains by successive or by simultaneous division depends upon the number of spindle fibers existing in connection with the first nuclear division. If there are enough fibers to form a cell plate completely across the cell, successive division results, while if the cell plate does not reach across the cell it is absorbed into the rest of the protoplasm, and the final division takes place simultaneously after the second nuclear division. In the onion the conditions are such as to indicate the necessity for the formation of new peripheral fibers. It will be remembered that at the time of the formation of the young cell plate there were but few radiating fibers visible, and those that could be seen were relatively short, with the exception of a few near the periphery of the central spindle. In the later stages, after the fibers had entirely disappeared from the central area, the same relations of spindle fibers in the peripheral portion of the spindle could be distinguished that have been previously described in the larch (*fig. 30*). This relation indicates that there has occurred a growth at least of the original radiating fibers, with the probable production of new ones.

There is another point of importance here. The fact that some of the longer peripheral fibers may be traced to the nucleus indicates a continuous production of new peripheral fibers from the nucleus as a center. The difference, then, between the larch and the onion is possibly as follows: in the former the extent of growth of the cell plate depends upon the number of existing fibers, while in the latter, where there is necessity for the

formation of a complete cell plate, the original fibers may be supplemented by the growth of new peripheral fibers. The cases in the larch in which the cell plate is not completed after the first nuclear division are possibly accounted for by the fact that the nucleus has begun to prepare for its second division, and consequently new fibers necessary for the completion of the cell plate are not produced.

Concurrent with the growth of the cell plate in extent and the disappearance of the fibers from a central portion of the spindle the nuclei come to lie nearer the cell plate (*figs. 6, 11*). This phenomenon has often been observed, but no adequate explanation for it has been suggested. From the appearance of the whole cell in the larch it would seem that such a migration of the nuclei is due to the mechanical pressure of the surrounding cytoplasm. While some granular cytoplasm has entered the region occupied by the spindle during the early stages, the later stages show very little addition until the cell plate is nearly complete and all of the spindle fibers have been nearly used up. The fibers seem to form a barrier against its ingress, so that it forms a denser layer around the whole spindle (*fig. 7*). It is possible, too, that the growth of the cell plate by the method previously described has displaced the cytoplasm around the spindle, and that such a displacement results in a greater pressure upon the nuclei.

The history of the carbohydrate material during the growth of the cell plate could not be followed with certainty. I find that it always appears plainly in connection with the spindle fibers which are forming the cell plate. In the older portions of the cell plate, however, where the spindle fibers had entirely disappeared, this substance was often indistinguishable, but in those cases where the cell plate was split, a young cell wall could nearly always be detected. It did not extend, so far as could be seen, the entire length of the cleft, but was only in the older portions (*fig. 31*). A peculiar case was sometimes found in the larch, where an incomplete cell plate appeared with a conspicuous cell wall formed between the halves of the older portion

lying in the cytoplasm, with some of the original fibers still attached to it while the daughter nuclei were again dividing. Whether this cell plate and wall were finally completed, I did not determine. The same stage of development of the cell plate was often observed, in which no cell wall could be detected. The significance of the presence of a cell wall without the previous appearance of the carbohydrate material has already been discussed.

The evidence for the splitting of the cell plate before the formation of the new cell wall need not be discussed in detail here. Treub, in his experiments upon living cells, showed that the splitting occurs, and the new wall is laid down between the separated halves before the cell plate has attained its full growth. My own observations confirm this fact (*figs. 19, 31*). But one point of difference should be noted between Treub's observations and mine. In Treub's figures the beginning of the process is represented on one side of the cell where the cell plate has reached the membrane of the mother cell, while in my preparations it may be clearly seen that the splitting begins in the central region of the plate and extends toward the periphery. The mechanics of such splitting is hard to explain. The fact that no evidence of it was found except where the spindle fibers had disappeared would indicate that the fibers have no part in the process. From his observations on *Fucus*, where the granules which form the cell plate divide before fusion, Strasburger suggested that the splitting may be the result of a similar process in the higher plants, *i. e.*, the separate cell plate elements may divide before they fuse. If such a division takes place the two parts remain so close together that no evidence of it appears until a much later stage. Where the cell plate elements are so conspicuous as they are in the larch, it is reasonable to expect to find stages showing their division if it really occurs. The lack of such stages seems to me to indicate that the division comes later, after the elements have fused into a continuous plate. If this be true, the problem is that of the splitting of a continuous protoplasmic membrane. Strasburger decided that

there is no change of a middle layer in the cell plate into a cell wall. This conclusion rests mainly upon the fact that the halves of the cell plate which appear after splitting are together equal in thickness to the original plate, *i. e.*, there has been no diminution of the substance of the cell plate during the process of splitting. That the cell wall is not a differentiated portion of the cell plate seems to me to be further shown by those cases in which portions of the separate halves of the cell plate appear with no cell wall between them (*figs. 30, 31*).

The splitting seems to be due primarily to a differentiation of the substance of the plate itself into two layers. Of what this differentiation consists is by no means apparent. It is hard to conceive of a layer of protoplasm becoming differentiated into two separate layers similar in all apparent respects to each other. To be sure, the two layers form the boundaries of separate cells, a fact which may be taken to indicate a possible chemical difference between them. This differentiation, in itself, moreover, would not account for the separation of the halves of the plate. A possible explanation for this latter phenomenon may be that there is secreted between the halves some non-stainable substance, perhaps cell sap, which serves to separate them. The apparent disappearance of the carbohydrate material before the cell plate halves appear separated may mean that there is a change in this substance, preparatory to its being deposited as a wall, of such a character that it does not take the same stain as before. It may be that it simply forms a less dense solution, and that in this form it is first deposited between the halves of the cell plate. The appearance of a stained wall would then mean that the substance had again become dense enough to take the stain. While the above explanations are purely hypothetical, I cannot see that they in any manner do violence to the observed facts. They are suggested only in order to help bring out some phases of the problem to be solved. It should be noted in this connection that the splitting of the cell plate is apparently not quite analogous to the longitudinal splitting of the chromatin thread, unless Strasburger's view that the granules divide before



they fuse into a cell plate is to be accepted. In the splitting of the chromatin thread we may easily imagine the process taking place by constriction, but in the case of a continuous layer of protoplasm like the cell plate such a process would be impossible. The more so when we remember, as my figures show, that the splitting begins in the central portion and extends outward.

The change in position of the cell plate during its growth is shown by *figs. 12 and 21*. The explanation of such a change is not obvious. The figures show that the nuclei do not move with the cell plate. Whether the cell plate ever changes from a true diagonal to a transverse plane I was unable to determine. The figures show only those cases where the plane in which the cell plate was first formed was not a true diagonal. Nemec<sup>39</sup> has shown that the position and form of the young spindle can be determined to some extent by means of pressure or tension exerted upon the tissues in which the dividing cells occur. It is possible in these cases that the conditions of pressure or tension upon the single cells have so changed by growth of some of the surrounding cells of the tissue as to bring about the change in position of the cell plate. The most obvious significance of the shifting of the cell plate is that the original position of the spindle cannot always be taken to determine accurately the ultimate plane of division of the cell.

#### CONCLUSIONS.

1. The most obvious and at the same time most important conclusion to be derived from the foregoing observations is that the division of the cell body is due to the activity of the kinoplasm of the cell. The splitting of the cell plate, or at least its differentiation into separate layers, is in the higher plants the essential act in the division of the cell body, for it is not until such a differentiation has taken place that there is a separation of the protoplasm of the mother cell into two distinct parts. That the cell plate is kinoplasmic has already been

<sup>39</sup> Ueber Kern u. Zelltheilung bei *Solanum tuberosum*. Flora 86: 214. 1899.

insisted upon by Strasburger in two of his recent papers.<sup>40</sup> The difference between Strasburger's observations and mine is that in the former only a small portion of any one fiber is used in the process of cell plate formation, while in the latter the substance of the whole fiber becomes transformed into a portion of the cell plate, *i. e.*, in the one case the cell plate is simply a product of the activity of the kinoplasmic fibers, the fibers themselves not being used in the process, while in the other case the cell plate is a result of a change of form of the substance composing the fibers. The identity in character of the substance of the cell plate and that of the spindle fibers has an important bearing on the relations of the different methods of cell division occurring in different groups of plants. The process of free cell formation in the embryo of *Ephedra*, as described by Strasburger, is an interesting case of kinoplasmic activity, in which the division takes place around a single nucleus instead of between two nuclei as in regular cell division. The fact that the membranes are formed simultaneously would show that this process goes on around all parts of the nucleus alike. It might be contrasted in this respect with the formation of ascospores, as described by Harper, in which the growth of the membrane is a progressive one and the nucleus is consequently modified in form to suit the necessity of kinoplasmic activity from one point alone. The method of division of the egg from the endosperm mother cell and the synergids, as described by Mottier<sup>41</sup>, presents a process intermediate between the method of free cell formation in *Ephedra* and that of regular cell division. Here, where there are two nuclei lying adjacent to one another, the cell plate is formed in connecting spindles between them, but where, as in the case of one synergid in Mottier's *fig. 22*, the fibers end blindly in the cytoplasm, a cell plate is formed in such fibers, following the method of *Ephedra*. In all of the above cases the formation of a membrane from the fibers is an established

<sup>40</sup> Ueber Cytoplasmastructur und Zellhäute.

<sup>41</sup> Ueber das Verhalten der Kerne bei der Entwicklung des Embryosacks und der Vorgänge bei der Befruchtung. *Jahrb. f. wissen. Bot.* 31: 123. 1898.

fact. The method of cell division by constriction of the plasma membrane, which occurs in many filamentous algæ, may be analogous to the growth of the plasma membrane as the cell increases in size, with the difference that in the former case the growth of the plasma membrane is localized and proceeds without the accompanying growth of the cell contents.

2. I have already indicated what seems to me to be the relation of the nucleus to the kinoplasm in the process of cell division. It is the center of the metabolic processes concerned in the production of the kinoplasm. Whether there is a transformation of some previously existing substance of the cell into the filar form of kinoplasm I have not been able to determine. From the two facts that no such transformation is visible and that a great many of the fibers are used up in the formation of a membrane, I think it probable that they are formed anew in each cell. That the chromatin is the real center for their formation is shown by the formation of new radiating fibers around the daughter nuclei in the onion during the diaster stage, and by the formation of a spindle around a single chromosome in the manner described by Juel for *Hemerocallis*. The history of the kinoplasm in a single cell of the higher plants would seem to be as follows. It is formed as fibers around the nucleus as a center. In this form the kinoplasm takes part in the process of nuclear division, and later divides the cell by a part of the fibers being transformed into a membrane which becomes in splitting the plasma membranes of the daughter cells.

In cases where cell division does not follow immediately upon nuclear division the filarplasm may be absorbed into the general protoplasm of the cell, either to reappear or to be formed anew when needed for cell division. The ultimate form of the kinoplasm of any one cell seems to be reached when it has become a plasma membrane. It is then concerned with the activities of the resting cell. The transformation of kinoplasm from the filar into the membrane form seems to show that the hypothesis of a permanent substance, which may be described

by the term "filarplasm," lately proposed by Strasburger,<sup>42</sup> is not well founded. To be sure there are, in a great many cells, spindle fibers that seem to take no part directly in the formation of membranes, but they apparently lose their filar form. The most permanent form of the kinoplasm seems to be of the membrane. It would seem better, then, to retain the older physiological term kinoplasm, since such a term would denote a substance having certain physiological properties without necessarily being limited to any one form. The conclusion reached by Kostanecki<sup>43</sup> of the permanency of the spindle fibers in the cell, and that they are always reproduced by division of the individual fibers, "omnis radius e radio," is, as Nemec<sup>44</sup> has pointed out, unsupported by the history of the fibers in plant cells. It would be impossible, moreover, to account thus for the manner in which the fibers are transformed into a membrane.

3. The relation of the carbohydrate material to the process of division would seem to show, as already stated, that the substance for the formation of the cell wall is held in a reserve form in the protoplasm before it is actually needed for the process of wall formation. The fact that it appears in connection with the spindle would suggest, as Farmer and Williams have shown, that the spindle fibers have for their substance a conductive function. These authors did not ascribe a formative function to the fibers because they did not find any cell plate. It is not impossible that the cell plate may have been entirely overlooked in this case. In some of my own preparations it was often very difficult to discover the young cell plate in the midst of the carbohydrate material.

If the above mentioned relation of the carbohydrate material to the spindle be taken in connection with the facts shown by Klebs,<sup>45</sup> and by Townsend,<sup>46</sup> that the presence of a nucleus is

<sup>42</sup> Zellhäute.

<sup>43</sup> *Op. cit.*

<sup>44</sup> Kerntheilung von *Allium Cepa*, 321.

<sup>45</sup> Beiträge zur Physiologie der Pflanzenzelle. Untersuch. a. d. botan. Institut z. Tübingen 2: 500. 1888.

<sup>46</sup> Der Einfluss des Zellkerns auf Bildung der Zellhäute. Jahr. f. wissen. Bot. 30: 484. 1897.

necessary for the formation of a cell wall, there would be some evidence for the hypothesis that the nucleus forms the cell wall substance.

The investigations described in the foregoing pages were begun at Lake Forest University under the direction of Professor R. A. Harper, whose helpful interest and valuable criticism have continued throughout their progress. The work was continued and practically completed in the botanical laboratory of the University of Michigan during the years 1897-8 and 1898-9. I wish here to thank Professors Spalding and Newcombe of the above laboratory for the liberal way in which material and equipment were provided, and for many helpful suggestions received. Professor Jacob Rheigard, of the department of zoölogy of the University of Michigan, very kindly allowed the use of the photographic apparatus of that department, and Dr. J. B. Johnston aided me greatly in making the photographs.

THE UNIVERSITY OF WISCONSIN.

#### EXPLANATION OF PLATES VIII AND IX.

Figs. 1-21 are reproductions of photographs made with the Zeiss photomicrographic apparatus. (Figs. 9 and 22 have been purposely omitted.) Figs. 23-31 are from drawings made with the aid of the camera lucida, Zeiss 2<sup>mm</sup> immersion objective, and compensation oculars 8, 12, and 18. The size has been reduced  $\frac{1}{3}$  in reproduction.

Figs. 1-10. *Larix*; pollen mother cells.

FIG. 1. Equatorial plate.  $\times 750$ .

FIG. 2. Early diaster showing first stages in preparation for cell plate formation; the central spindle is differentiated into three zones.  $\times 1200$ .

FIG. 3. Later than preceding; central spindle alike throughout.  $\times 1200$ .

FIG. 4. Cell plate elements just forming.  $\times 1200$ .

FIG. 5. Young cell plate has begun to grow in area; connecting fibers are beginning to disappear.  $\times 1200$ .

FIGS. 6-10. Later stages in the growth of the cell plate; see explanation in text.  $\times 1200$ .

FIGS. 11-21. *Allium Cepa*; cell of growing root-tip.

FIG. 13. Equatorial plate; spindle fibers arranged into strands or bundles.  $\times 1500$ .

FIGS. 14-15. Metaphases showing granular appearance of region between the groups of daughter chromosomes.  $\times 1500$ .

FIG. 16. Stage corresponding to *fig. 2*.  $\times 1500$ .

FIGS. 17-19. Later stages showing the equatorial zone containing carbohydrate material.  $\times 1500$ .

FIGS. 18-19. Formation of cell plate in the midst of the zone of carbohydrate material.  $\times 1500$ .

FIGS. 20 and 21. Growth of cell plate and disappearance of spindle fibers.  $\times 1500$ . See also *fig. 11*.

FIGS. 12 and 21. Stages showing change of position of cell plate from a diagonal to a transverse position.  $\times 1500$ .

FIG. 23. *Larix*; showing arrangement of spindle fibers and differentiation of cytoplasm into layers.  $\times 3000$ .

FIG. 24. *Larix*; details in relation of radiating fibers to the connecting fibers in a stage represented by *fig. 3*.  $\times 4500$ .

FIG. 25. *Allium*; portion of spindle in late metakinesis, showing relations of fibers to one another and distribution of granules in the spindle.  $\times 4500$ .

FIG. 26. *Larix*; two radiating fibers lying adjacent to the central spindle and crossing in such a way as to appear fused.  $\times 4500$ .

FIG. 27. *Larix*; different stages in the transformation of the substance of a spindle fiber into a portion of the cell plate.  $\times 4500$ .

FIG. 28. *Allium*; relation of the connecting fiber to the carbohydrate material at the equator.  $\times 3000$ .

FIG. 29. *Allium*; cell plate elements formed on the connecting fibers in the midst of the carbohydrate material.  $\times 3000$ .

FIG. 30. *Allium*; late stage in the growth of the cell plate, showing the relation of the outer radiating fibers to the growing portion of the plate.  $\times 4500$ .

FIG. 31. *Allium*; cell plate with young cell wall showing in the cleft where the cell plate is split.  $\times 3000$ .

ERRATUM: on p. 85, line 16 from top, *fig. 10* should read *fig. 13*.

## PHYSIOLOGICAL OBSERVATIONS ON SOME PERENNIAL HERBS.

A. RIMBACH.

(WITH PLATE XIII)

### I.

*Arisaema Dracontium* (L.) Schott.—During winter *Arisaema Dracontium* consists of a stem-tuber, the growing point of which, covered by several scale leaves, lies at about 5<sup>cm</sup> below the surface of the earth. It is devoid of roots. Early in April the bud begins to elongate upwards, and at the same time from fifteen to thirty roots break out in a ring like zone from the base of the bud (*fig. 4*). They appear almost simultaneously and grow horizontally, radiating in all directions from their point of origin. They are 1 to 1.5<sup>mm</sup> in diameter throughout their entire extent, and attain a length of more than 20<sup>cm</sup>. After a time some of them become somewhat transversely wrinkled at the base, because they undergo there a slight longitudinal contraction. At the end of April the scale leaves protrude from the soil. They are tightly appressed to each other and enclose a hollow, in which the foliage leaf develops (*fig. 6*). In the latter the end leaflet is vertically extended, the lateral leaflets are bent downwards, and the blades of all are involutely rolled up. After having reached the surface of the earth, the scale leaves stop their growth, and at the end of April or beginning of May the foliage leaves and inflorescences break out from their coverings and unfold in the air. In the course of May the inflorescence opens. About the same time a second set of roots is formed immediately above the first one (*fig. 5*), but there is a striking difference between the two. The roots of the second set are thicker, about 2.5<sup>mm</sup> in diameter, and taper towards the end. They grow from their origin vertically downwards or nearly so, and pass over into a horizontal direction only at their thin end

portion. They undergo very soon a considerable longitudinal contraction in the thickened basal portion, which is usually 2 to 4<sup>cm</sup> long. After having attained a certain intensity, this contraction manifests itself externally by a shriveling of the root surface. I noticed, however, that these roots of the second set are not in all individuals developed in the same degree. In specimens, for instance, which were located at a considerable depth, these roots differed very slightly from those first formed. On the other hand, in individuals which were placed very superficially I found the roots exceptionally thick and exceedingly numerous.

The contraction amounts to about 40 per cent. within the space of 5<sup>mm</sup> in the swollen basal portion. It diminishes in intensity towards the thin terminal portion, where no contraction at all takes place. The whole contraction of the root amounts to about 15<sup>mm</sup> or even more, the contractile region having originally an average length of about 5<sup>cm</sup>. Of these facts I satisfied myself by marking and measuring the roots during their development in the earth. For this purpose the plants were cultivated in specially constructed culture-cases, furnished with lateral windows, which could be removed thus permitting access to the roots. The active contractile tissue is here, as in other roots of similar structure, the cortical parenchyma. The central axis of vascular bundles with the endodermis is passively contracted, and the same holds good for the epidermis and exodermis and one or two layers of parenchyma immediately below the latter. These passive layers of the outer cortex very soon become folded, and form transverse wrinkles. During contraction, which process lasts in the whole root about three or four weeks, the shortening cortical parenchyma cells elongate very much radially. The root, however, does not grow in diameter, but the outermost layers of the active parenchyma after a time collapse and become tangentially compressed by the inner ones, so that finally a rather wide area of crushed cells is found surrounding a few layers of the still turgescient innermost cells. In the endodermis, as well as in the exodermis; there is also a remarkable



result of the root contraction noticed. The radial longitudinal walls of their cells, being quite straight at the beginning, as contraction sets in become marked with wavy foldings. In the endodermis the waves are strongest in that longitudinal band of the wall which corresponds to the well-known dark spot on the cross-section.

As each root is fastened at its tip to the earth and at its base to the tuber, in consequence of the contraction a tension is set up in it, and the root must give way at the point less firmly fixed. As a matter of fact, mostly the base of the root moves towards the tip and pulls the tuber with it. Thus, by the combined action of all the roots, since all in their basal part point steeply downwards and differ but little in direction, the tuber is drawn down into the earth a certain amount every year.

Thus the contractile roots determine to a great extent the position of the tuber. The latter is found sometimes upright, sometimes lying horizontally, not seldom even turned over with the bud directed downwards, so that the leaf-stalks and stems have to make a strong curvature in order to attain an upright position. The situations are largely due either to a uniform or one-sided pull of the roots. I am not able to say with certainty which direction of growth the tuber would take up by itself, and whether its behavior in this respect would be the same under different external conditions. The annual prolongation of the tuber is in larger individuals from 6 to 10<sup>mm</sup> (*fig. 6*), and if the tendency of growth were always upright, the dragging action of the roots would of course be of essential importance for keeping the growing point in place and preventing its emerging from the earth.

All the roots are furnished with root-hairs, and form later a few branches of the first order in their terminal portion. At the time of the withering of the leaves all the roots die off, and their scars are then found in a ring-like zone on the surface of the tuber (*sc, fig. 4*).

In the axils of the scale leaves lateral buds originate, four or five every year, which persist after the leaves which support

them have disappeared, and are found in the fall of the same year as lateral protuberances upon the fully developed portion of the tuber (*bd*, *fig. 6*). During the next spring they enlarge considerably, and later, as that portion of the tuber on which they are inserted becomes emptied and dies off, they are set free. As a rule, they do not yet develop a leaf and roots in the same year, but remain dormant and sprout only in the second year after that in which their supporting leaves were vegetating. The tuber always contains but one year's growth in a fully developed state.

The leaves and roots perish in July or August, and soon after the berries ripen. During the germination of the seed, which takes place in the following spring, the cotyledon, growing downwards, elongates so much that the growing point of the stem of the seedling is located 8 to 10<sup>mm</sup> below the seed (*fig. 1*). The upper end of the cotyledon, which remains in the seed, swells up so as to form an ellipsoid sucker, which finally becomes about 5<sup>mm</sup> long and displaces in part the emptying endosperm. While this is taking place the first foliage leaf sprouts out and penetrates the earth with a knee-like nutation of its stalk (*l*, *fig. 1*). Already the first root of the seedling, reaching 3-5<sup>cm</sup> in length and 0.75<sup>mm</sup> in thickness, is a little contractile at its base, although it does not become wrinkled. During the subsequent development the little stem of the seedling swells up, chiefly below the insertion of the cotyledon, forming a little tuber; out of the latter, during the first year, two, or mostly three, seldom four, adventitious roots are formed (*fig. 2*). Each one of these grows longer and thicker than its predecessor, and develops also a longer contractile region. The fourth root, for instance, is usually about 10<sup>cm</sup> long and 1.5<sup>mm</sup> thick; its contraction amounts within the space of 5<sup>mm</sup> to 40 per cent.; its total contraction amounts to about 10<sup>mm</sup>. I observed in various specimens that by the work of the roots the little tuber was drawn down from 8 to 10<sup>mm</sup> during the first vegetative period. So we find, at the end of the first period of growth, the terminal bud of the plant from 15 to 20<sup>mm</sup> deeper than it was located

in the seed before germination (*fig. 3*). In the following years this migration of the plant downwards continues in a similar way. The alternation of roots mentioned above shows itself from the second year onward.

While the occurrence on one and the same plant of two kinds of roots differing in form and function is not very rare, the formation of these at different times, as in *Arisaema*, has so far been found only in a few species. I have noted the same fact, for instance, in *Allium ursinum* L., *Fritillaria Meleagris* L., *Scilla bifolia* L., and some other monocotyledons.

*Arisaema triphyllum* (L.) Torr. resembles perfectly *A. Dracontium* in the behavior of the underground organs.

The contraction of the roots seems to play a great part in another American species of Araceæ, in *Spathyema foetida* (L.) Raf. In this plant a stem-tuber is formed, which grows vertically upwards, dying off gradually at its lower end. This tuber attains 10<sup>cm</sup> in length and 5<sup>cm</sup> in thickness, and comprises the products of several years. It forms yearly about fifteen roots near its upper end. These grow obliquely downwards, tapering toward the tip, and produce from their thin end portion lateral rootlets of the first and second orders. They live several years, so that about sixty to seventy of them are found in one plant. All the roots are contractile, and apparently prevent the emerging of the tuber from the ground. This has been assumed also by Foerste, who found the seedlings germinating within an inch of the surface of the ground and the top of the root stock several inches below the surface. I found the seeds germinating on the surface of the soil and the growing point of the tuber in several larger specimens at a depth of about 10<sup>cm</sup>.

Just the same phenomenon may be observed in *Hypoxis hirsuta* (L.) Coville. This plant also has an upright growing stem-tuber attaining 3<sup>cm</sup> in height and 2<sup>cm</sup> in diameter. All its roots, which start from it in a ring-like belt, are more or less contractile in their thickened basal portion, which becomes transversely wrinkled, and as they grow steeply downwards they are enabled

to drag the tuber vertically into the ground. I found the growing point of older specimens mostly in a depth of 3<sup>cm</sup>.

In *Trillium sessile* L. I found most of the roots possessing a strongly contractile swollen basal portion, which soon becomes wrinkled. In this species the tuber very often by the power of the roots is drawn into a horizontal or even downwardly directed position.

*Mesadenia tuberosa* (Nutt.) Britton, a composite growing on wet places of the prairie, has a stem-tuber about 2<sup>cm</sup> in length, which yearly grows from 10 to 12<sup>mm</sup> vertically upwards, while it dies off in the same proportion at its lower end (*fig. 7*). The tuber comprises the products of two years, separated by a constriction. Every new member of the tuber, in May after its formation, sends out about twelve roots, originating in a simple ring. These roots grow obliquely downwards and attain a length of more than 30<sup>cm</sup>. They are at the beginning about 2<sup>mm</sup> thick, but grow secondarily in thickness, attaining at least a diameter of 4–5<sup>mm</sup>. They exhibit considerable contraction, and pull the stem as much back into the ground as it elongates upwards. The roots seem also to show reserve material. They persist through two vegetative periods and form later numerous long but very thin lateral rootlets, branching sparingly in their turn. There is a vegetative multiplication by lateral buds, which become isolated by the decay of the mother tuber.

The species of *Arisaema*, *Spathyema*, *Hypoxis*, *Trillium*, and *Mesadenia* mentioned above can therefore be classed together in one group, characterized by possessing a rhizome growing more or less vertically upward, which becomes drawn down by contractile adventitious roots. To the same group belong also the following species: *Hyacinthus candicans* Baker, *Lilium Martagon* L., *Allium ursinum* L., *Succisa pratensis* Moench, and *Plantago major* L.

## II.

*Erythronium albidum* Nutt.—During summer the bulb of *Erythronium albidum* lies hidden in the earth without aerial organs and without roots. I found the growing point of flowering bulbs,

of which I examined about twenty-five specimens in the month of April, mostly between 15 and 16<sup>cm</sup> distant from the surface of the earth, the extreme cases being 11 and 20<sup>cm</sup>. The sterile specimens, the bulb of which as a rule is smaller, were encountered always at a less depth. Of the about 200 specimens of this latter kind which I examined, the larger ones were found at an average depth of 9.5<sup>cm</sup>, the extremes being 5 and 15<sup>cm</sup>, and the smaller ones at an average depth of 7.5<sup>cm</sup>, the extremes being 4 and 13<sup>cm</sup>. I took into consideration only those individuals which were found on an even ground and apparently had not suffered any considerable disturbance. Hence the smaller plants, as a rule, are found in a more shallow position than the larger ones.

In the late fall—the exact time I am unable to give—the roots break out from the stem portion of the bulb. They appear all at once, their number being in the smallest specimens from four to ten, in the largest from twenty to forty. They are about 0.75<sup>mm</sup> thick, uniform throughout, become about 15<sup>cm</sup> in length, and never branch. In the specimens I examined root hairs were not present. These roots do not exhibit any contraction. Their direction of growth varies very much, and seems to be largely influenced by local factors.

At the beginning of April the leaves emerge from the ground, penetrating the earth with the cartilaginous point of the erect inwardly convolute blade. The young specimens develop only one leaf, the older ones a stem with two leaves and with or without a flower. With the development of the aerial organs the emptying of the old bulb-scales and the formation of a new bulb begin. The new bulb may be formed close to the old one, or may be removed from it to a more considerable distance by means of a runner. Of 200 sterile individuals I examined, 114 had formed a runner, the rest not, and of 25 flowering individuals only three had formed runners.

The runner is solid in its basal half, but in its terminal half it contains a narrow, longitudinal channel, the termination of which on the surface is found usually a little below the middle of its

length on the side opposite the roots. In those sterile specimens which do not form a runner the corresponding opening is found on the same side of the bulb at the base of the foliage leaf, about 5<sup>mm</sup> from the roots upwards. In both cases the opening leads to a hollow space, near the end of which the new bulb is formed. In the sterile individuals mostly two runners are formed by each bulb, the one being about twice as long as the other and also thicker. The longer runner reaches an average length of about 12<sup>cm</sup>, with a diameter of 2-3<sup>mm</sup>, the longest I found being 17<sup>cm</sup> in length. In flowering specimens I found the runner 3-5<sup>mm</sup> thick and 8<sup>cm</sup> long, but I do not know the definite length it may attain. Where the new bulb is formed close to the old one, one finds instead of the smaller runner only a little bud. The smaller runner, as well as the little bud mentioned, may be found sometimes at the right, sometimes at the left side of the main bulb.

Without entering into morphological considerations about the parts of stem and leaves which take part in the formation of these structures, we will consider only their physiological behavior. At the end of April the elongation of the runner stops, and the new bulb enclosed in its point begins to swell up. At this time the old bulb-scales are completely emptied, but starch grains are found in the whole extent of the runner and in large quantity in the new bulb. During May the development of the new bulb is accomplished, and at the end of the month the foliage leaves, the old bulb, and the runner die off, so that at the beginning of June the new bulb is isolated. It remains in a dormant state until the fall of the same year, when the above described cycle recommences.

The difference between those specimens which separate the new bulb from the old one by a long runner, and those which form it close to the old one, lies only in the relative length of the stalk uniting the stout stem-portions of the two bulbs. Those stem-portions in which the leaves are inserted and from which the roots start are rich in vascular bundles, and their remnants may be preserved several years. Sometimes two, three,

or four of them are found still attached to a bulb, indicating the places where the plant had rooted in former years. In case no runner is formed, the bulb advances from 2 to 6<sup>mm</sup> every year, this being the distance of the points of rooting of the subsequent bulbs. Through the formation of a runner, the distance of the subsequent bulbs amounts to 3 to 10<sup>cm</sup>, being as a rule somewhat smaller than the length of the runner, as the latter usually is curved.

By the growth of the stalk uniting the subsequent bulbs, whether this stalk be short or long, the plant may suffer a dislocation both in horizontal and vertical direction. In both cases the position of the rhizome is somewhat singular, the side from which the roots are formed being turned upwards, and the side on which the bud arises downwards. Where a short stalk is formed, it is directed obliquely downwards and advances the plant a few millimeters in this direction. Where a runner is developed, it grows horizontally at the beginning, but later turns more or less vertically downwards, thus placing the new bulb several centimeters lower. I found in an examination of about 100 specimens the new bulb of the main runner on an average 4.6<sup>cm</sup> deeper than the old one, while the bulb of the smaller runner was sunken but half as deep. The extreme cases of sinking observed in the larger runner were 1<sup>cm</sup> and 10<sup>cm</sup>, in the smaller one 0<sup>cm</sup> and 6<sup>cm</sup>. In full-grown flowering specimens a runner seems to be formed but rarely, as I found in most of them the remnants of several years' growth close to the actual bulb, the plant advancing horizontally about 6<sup>mm</sup> every year. But the young, small individuals also, even in a rather shallow position, do not form a runner every year. Specimens of this latter kind which I found had formed no runner during three years; others, which had developed a runner this year, had been devoid of it in the preceding year, as was shown by the remnants of the last year's bulb; others, furnished with a runner the present year, seemed to have formed one also in the foregoing year, since not the slightest indication of an old bulb was present in their neighborhood.

I do not know what rule may exist in this alternation or by what factors the formation of a runner may be determined. Nevertheless, from my notes it seems that a shallow position of the plant favors the development of a runner. Probably all the shallow, small individuals are derived from seedlings, germinated near the surface of the soil. Their mode of growth must bring them gradually into the depth of the full-grown individuals. By the yearly duplication of their bulbs a rapid vegetative multiplication takes place.

*Erythronium mesachoreum* Knerr.—This plant, which resembles *E. albidum* very much, inhabits the open prairie. I found the growing point of the bulb in about twenty-five full-grown individuals which I examined between 9 and 13<sup>cm</sup> distant from the surface of the earth, the average depth being about 11<sup>cm</sup>. In this species the small, sterile individuals occupy a more superficial position, being found at a depth of from 3 to 8<sup>cm</sup>. They apparently have originated from seeds. In the early part of April the formation of the new bulb begins. In the young shallow individuals I always found the new bulb removed from the old one by a runner, but the latter is short, the longest I saw being about 20<sup>mm</sup> in length, and it grows from the beginning vertically downwards, thus placing the plant every year from 3 to 20<sup>mm</sup> deeper (*fig. 8*). In this species I never noticed the formation of more than one runner by one bulb. The result of this manner of growth is that the formations of the subsequent years are arranged in a vertical row, and I found, in fact, the remainders of the products of five years located in this way above the living bulb; the leaf of the present year making its way through the long channel formed by all the dead integuments (*fig. 9*). By such a movement of growth the plant finally reaches a depth beyond which it does not advance. I found, indeed, that in the old full-grown individuals, situated at a depth of about 11<sup>cm</sup>, the bulb grows no longer downwards, but horizontally or nearly so, the new bulb rooting about 4<sup>mm</sup> laterally from the old one.



The observations on *Erythronium mesachoreum* as well as *E. albidum* were made in the neighborhood of Lincoln, Neb.

So far as the physiological behavior of the rhizome and roots is concerned, *Lilium superbum* and *Medeola Virginiana* agree almost perfectly with *Erythronium*.

*Lilium superbum* L. has a horizontal rhizome, in which stem and leaves are fleshy, and participate in the same degree in the storing of reserve material. Each year's growth of the rhizome is in full-grown specimens about 4<sup>cm</sup> long, forming at first a runner-like stem portion beset with a few small fleshy scales, and at last a stout, bulb-like structure with numerous crowded fleshy scales, from the middle of which the aerial stem arises. From the under side of this terminal bulb-like portion of the rhizome, just at the place where the aerial stem originates in June, about ten thin roots grow out horizontally, radiating towards all sides and sparingly forming lateral branches of the first order. They do not exhibit any contractility, and last but one year, the rhizome dying off from behind very quickly. I found the growing point of the rhizome in full grown specimens between 7 and 10<sup>cm</sup> below the surface of the earth. The plant must reach and keep this depth, as the roots are quite inactive, by the movement of growth of the rhizome itself. This behavior, which some other American species of *Lilium* seem to share, is entirely different from that of *Lilium Martagon* and other species of the Old World, in which the bulb grows vertically upwards, and is drawn down by strongly contractile roots.

*Medeola Virginiana* L. has a horizontally creeping tuberous rhizome, representing but one year's growth in the fully-developed state. The hibernating, tuber-shaped, end portion of the rhizome bears about twenty or twenty-five thin, thread-like roots, which attain 15<sup>cm</sup> in length and produce numerous branches of the first and a few of the second order. These roots are not contractile, and radiate in all directions from the rhizome, meandering very much in their course. On even soil I found the rhizome growing in a depth varying from 1 to 4<sup>cm</sup>. Also here the movement is due to the rhizome alone, the roots taking no part in it.

The roots of *Erythronium albidum* and *E. mesachoreum*, *Lilium superbum*, and *Medeola Virginiana* have only a nutritive function. They are of no great importance in fixing the plant in the earth, nor do they exert any strain upon the parts from which they start, nor do they store any considerable quantity of reserve material. In contradistinction to those of *Arisaema*, *Spathyema*, *Trillium*, and *Hypoxis*, we find in these roots but little cortical parenchyma, no compressed cortical cell layers, no wrinkling of the root surface, and a total absence of wavy foldings in the longitudinal walls of their endodermis and exodermis. The species of *Erythronium*, *Lilium*, and *Medeola* above mentioned are representatives of a type of geophilous plants in which the rhizome, by its manner of growth, seeks and keeps a certain depth in the ground, without any help of the roots. *Dentaria bulbifera* L., *Paris quadrifolia* L., *Colchicum autumnale* L., *Orchis mascula* L., and *Platanthera montana* Reichb. f. are species which belong to the same type.

### III.

Many perennial herbs develop a long tap root, which becomes more or less thick and fleshy, and filled with reserve material. To this group belong the following species: *Kuhnia eupatorioides* L., *Lacinaria punctata* (Hook.) Kuntze, *Grindelia squarrosa* (Pursh) Dunal, *Nothocaleis cuspidata* (Pursh) Greene, *Kuhnistera candida* (Willd.) Kuntze, *K. purpurea* (Vent.) MacM., *Psoralea esculenta* Pursh, *Astragalus crassicaulus* Nutt., *Peucedanum foeniculaceum* Nutt., *Callirrhoe alceoides* (Michx.) A. Gray, *Delphinium Carolinianum* Walt., *Aquilegia Canadensis* L., *Asclepias tuberosa* L., *Lithospermum angustifolium* Michx., *Physalis longifolia* Nutt., and *Allionia nyctaginea* Michx.

In many of these plants the contractility of the root is an important feature. I have noted this phenomenon in *Allionia nyctaginea*. In seedlings of this plant it can be observed easily that the base of the cotyledons, which at the beginning finds itself above the ground, after some weeks disappears under the surface of the earth. That this is really due to the contraction of the root I satisfied myself by marking the root of young

specimens, grown in a special culture case, from their base with lines of India ink 5<sup>mm</sup> apart. These 5<sup>mm</sup> spaces shortened to 4<sup>mm</sup> each within six weeks, which equals a contraction of 20 per cent. At the same time the uppermost line, that next the root base, moved about 6<sup>mm</sup> downwards, placing the base of the shoot so much deeper.

Also in *Aquilegia Canadensis* I observed a considerable shortening of the root, which results likewise in drawing down the growing point into the earth. In seedlings of *Aquilegia vulgaris* L. I noticed that the growing point of the stem, which immediately after germination stood 8<sup>mm</sup> above the ground (*fig. 11*) was found at the end of the first summer, in consequence of the contraction of root and hypocotyl, about 6<sup>mm</sup> below the surface of the earth (*fig. 12*). The upper lateral roots become bent down during this process in a very characteristic manner, (*r*<sup>2</sup>, *fig. 12*). The transverse wrinkling of the surface in the older roots of *Aquilegia* and *Allionia* is due to the same cause, and corresponds to the folding of the roots of *Arisaema*, *Hypoxis*, and others.

There can scarcely be any doubt that in *Lithospermum angustifolium*, *Nothocalais cuspidata*, *Peucedanum foeniculaceum*, *Astragalus crassicaulus*, *Kuhnistera candida*, and *K. purpurea* the root is also contractile, although I have not had the opportunity to measure it directly. This seems to be indicated by the following points: the undulating course of the innermost vascular bundles, the transverse wrinkling of the outer bark, the disturbed position of the upper lateral roots, and finally the position of the growing point below the surface of the earth, in spite of the continual prolongation of the perennial stem-portion towards above. Also in *Kuhnia eupatorioides* and *Grindelia squarrosa* contraction seems to occur, although in a less degree, while in *Lacinaria punctata* it possibly may not exist at all. In pieces of roots of *Lithospermum angustifolium* and *Kuhnia eupatorioides* lying in water, I observed after two days a considerable shortening.

In addition I might say something concerning *Lacinaria squarrosa* (L.) Hill, which, although resembling *L. punctata* very

much in its aerial organs and even in its germination, differs considerably in the development of its underground organs. *Lacinaria squarrosa* has an underground roundish tuber about 3<sup>cm</sup> high with four lateral swellings, a partition that seems to correspond to the tetrarchic arrangement of the vascular bundle in the primary root. I am not able to say, however, whether the tuber is formed only by the basal portion of the primary root, or whether the hypocotyl also or the stem take part in it. At all events, the terminal portion of the main root does not persist, but is replaced by new lateral roots starting from the tuber. Those roots originate in the older plant in four groups of from five to fifteen situated on the four swellings of the tuber (*fig. 13*). They break out from the tuber all at once in the latter part of April at the time when the first leaves appear. They are white, soft, somewhat thickened in their basal portion to an extent of 2 or 3<sup>cm</sup>, attaining here a diameter of 2<sup>mm</sup> and tapering very rapidly to a diameter of only 0.5<sup>mm</sup>. They grow at the beginning almost vertically downwards, reach about 50<sup>cm</sup> in length, and form very thin branches of the first and second orders, with numerous long root-hairs. The thickened portion of the root consists at the beginning mostly of soft, thin-walled tissue and is contractile, exhibiting according to my measurements in the culture case a shortening of 6–10 per cent. within a space of 5<sup>mm</sup>. The whole root probably never shortens more than from 2 to 4<sup>mm</sup>. The contraction ceases after four or five weeks of growth of the root, and during this time the characteristic wavy foldings in the walls of the endodermis and exodermis make their appearance. Later the root becomes more rigid on account of the development of thick-walled cells in the central axis. At the beginning of October, when the fruits are ripe and the aerial shoots begin to die, the roots perish, and during winter there are no living roots on the plant. In old specimens of *Lacinaria squarrosa* the roots probably cannot produce a movement of the tuber, while in younger plants it possibly might happen. But they help apparently to hold the plant in place by the strain they exert. This contrivance seems to be useful, since

perennial organs of attachment are wanting, and since the tuber is very superficially located and the aerial shoot is relatively high, heavy, and exposed to strong winds.

*Physalis longifolia* Nutt. does not show any contraction of its long fleshy tap root, but reaches a considerable depth in quite another way. The plumule of the seedling is raised 5 to 10<sup>mm</sup> above the ground and transforms itself afterwards into a long shoot. The primary root grows vertically downwards, sending out numerous thin lateral rootlets, and becomes subsequently thick, fleshy, and filled with starch. Very soon on its surface adventitious buds appear, some at the upper end at the limit of the hypocotyl, rarely on the hypocotyl itself, others deeper down at a distance of 6 or 8<sup>cm</sup> from the surface of the earth. At the close of the vegetative period the upper part of the plant dies down, but the root, at least a part of it, with its buds, hibernates, and at the beginning of the next year one or more of the buds, now much deeper than the plumule was, grow out by means of the reserve material stored up in the root. In older specimens I found the root 1<sup>cm</sup> thick and extending to a depth of more than 50<sup>cm</sup>.

The seedling of *Asclepias Cornuti* Decne shows a development of root shoots quite similar to that of *Physalis longifolia*.

*Delphinium Carolinianum*, *Callirrhoe alceoides*, and *Nothocaleis cuspidata* are distinguished by the peculiar phenomenon that in older specimens the root is slit into several longitudinal cords in consequence of the dying off of certain tissue portions. I found the root of *Delphinium*, for instance, divided into eight cords, forming a circle round a central hollow space and connected above and below. In *Nothocaleis* the root at a length of 12<sup>cm</sup> becomes variously pierced and divided into two, three, or four longitudinal cords, each of them about 6<sup>mm</sup> thick, united lattice-like at different heights. This fission of the root is not caused by simple decay of the older tissue, but is due to a peculiar mode of growth in thickness. The details of the processes concerned deserve closer study. A corresponding phenomenon has been observed and studied by Fost in *Gentiana cruciata* L.,

*Corydalis nobilis* Pers., *C. ochroleuca* Koch, *Salvia pratensis* L., *Aconitum Lycoctonum* L., and *Sedum Aizoon* L. I observed it in *Scabiosa arvensis* L. and saw a fission into four cords also in the subterranean stem of *Gentiana puberula* Michx.

In addition, I have noticed in *Nothocalais cuspidata* a daily opening and closing of the flower heads. My observations were made between May 1 and 15, in sunny, moderately warm, windy weather on the prairie near Lincoln, Nebraska, where this plant grows naturally. The heads begin to open at 7 A. M., and are at 9 A. M. fully expanded. They remain so till 3 P. M., at which time they begin to close again. At 4:30 P. M. they are all closed, and they stay so during the night.

All the plants mentioned, furnished with a deep tap root, are confined to the place they occupied when germinating. Besides, they lack vegetative multiplication, their only means of propagation being by seeds. Perennials of this type are exceedingly numerous on the prairie. Nevertheless, other forms occur also, like *Helianthus scaberrimus*, with long, horizontal rhizomes, by means of which they are enabled not only to change their place, but also to multiply vegetatively.

*Helianthus scaberrimus* Ell., as found in early spring, consists of a subterranean shoot 2 to 4<sup>cm</sup> long, horizontal or ascending at its end, furnished with about twelve long rigid roots, directed obliquely downwards and forwards. At the end of April the terminal bud of the rhizome leaves out, forming immediately long internodes, the first of which bear scale leaves. In the middle of May, from the stem nodes between the roots and from the axils of the scale leaves above them, six to ten white runners start. These take up a horizontal direction and continue growing till August, attaining a length of from 50 to 100<sup>cm</sup>. Like the leaves of the aerial stem, the scales of the rhizome are opposite, the youngest ones covering the growing point and protecting it during its way through the earth. The runner is 2 to 3<sup>mm</sup> thick and consists of about twenty internodes, the longest of which may measure 8<sup>cm</sup>. The last four or five internodes of the runner are very short, and this stout end-portion at the

middle of September gives rise to new roots, while its terminal bud, having stopped its growth, remains quiescent until next spring. At the middle of November, when the aerial shoot has died down, the runner begins to decay from its base, while the roots have reached a length of about 20<sup>cm</sup> and are still growing. During winter the runner decays, only its end portion with the roots remaining alive, and the plant assumes again the form in which we find it in the spring.

LINCOLN, NEBRASKA.

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#### EXPLANATION OF PLATE XIII.

All figures are drawn from nature, and, with exception of *fig. 10*, are natural size. The horizontal dash-lines indicate the surface of the earth.

FIG. 1. Seedling of *Arisaema Dracontium* still in connection with the seed; *s*, seed; *c*, cotyledon; *l*, first leaf; *r*, first root.

FIG. 2. The same, near the end of the first period of growth, having developed all its roots; *z*, tuber; *r'*, contractile adventitious roots.

FIG. 3. The same, in the resting state after the first period of growth.

FIG. 4. Small specimen of *Arisaema Dracontium* in April, forming the first set of roots;  $r^1$ , thin roots of the first set;  $sc$ , scars from the roots of the preceding years.

FIG. 5. A similar specimen, in May, forming the second set of roots;  $r^1$ , thin roots of the first set;  $r^2$ , thick contractile roots of the second set.

FIG. 6. *Arisaema Dracontium*, leafing out; longitudinal section;  $sc$ , scars from roots of the preceding years;  $sk$ , remnants of the skin of tuber portions emptied in former years;  $bd$ , lateral bud; the shaded part of the tuber is to be emptied this year.

FIG. 7. Tuber of *Mesadenia tuberosa*, in May; longitudinal section;  $r^1$ , roots formed in the present year;  $r^2$ , roots formed in the preceding year.

FIG. 8. Young descending specimen of *Erythronium mesachoreum* in April, with the runner developed;  $b$ , bulb;  $l$ , leaf-stalk;  $r$ , roots;  $R$ , runner.

FIG. 9. Young sterile descending specimen of *Erythronium mesachoreum* with its remnants of the five preceding years.

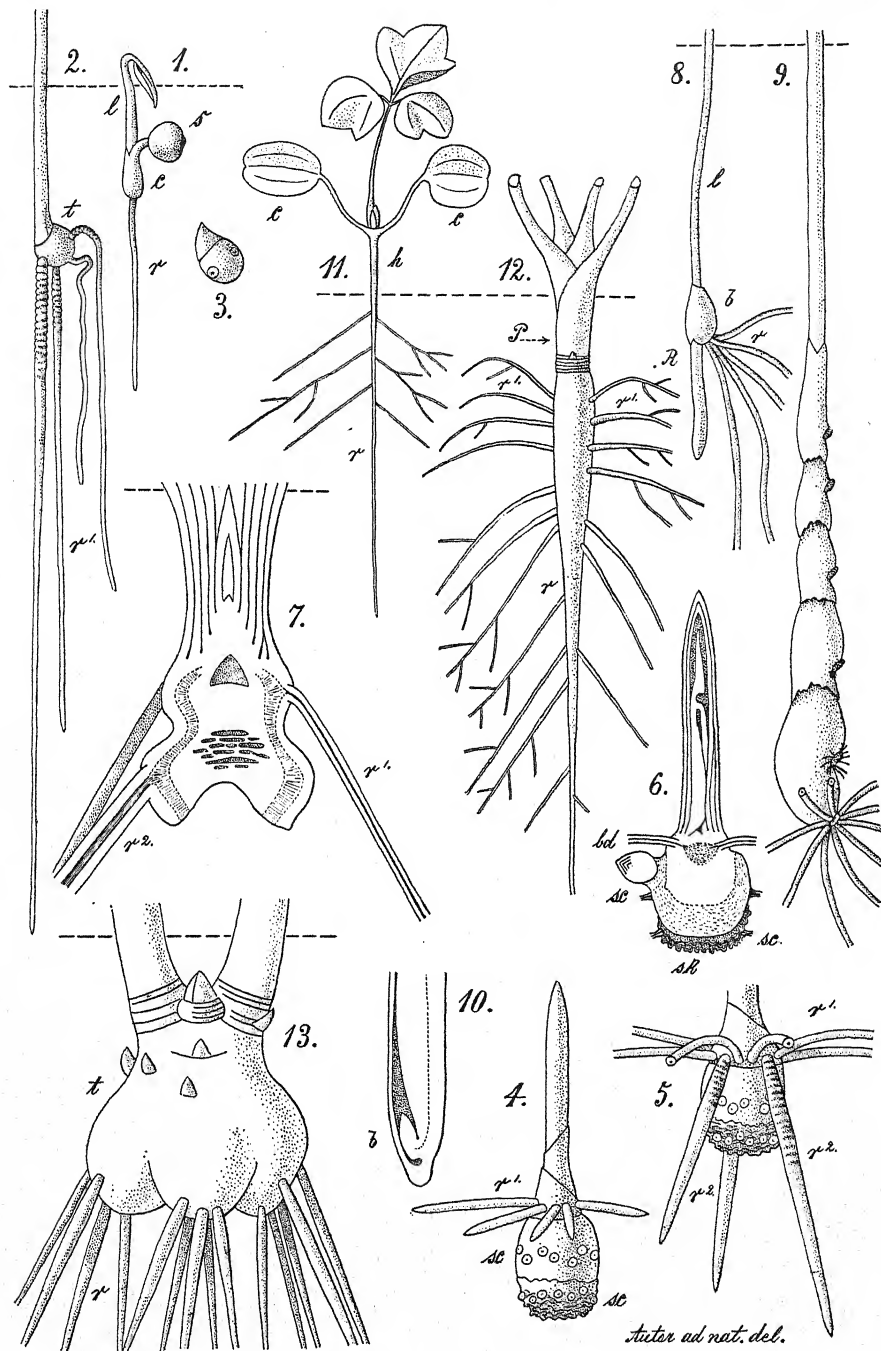
FIG. 10. Tip of the runner of *Erythronium mesachoreum*; median longitudinal section;  $b$ , new bulb.  $\times 5$ .

FIG. 11. Seedling of *Aquilegia vulgaris*, soon after germination;  $h$ , hypocotyl;  $c$ , cotyledons;  $r$ , primary root.

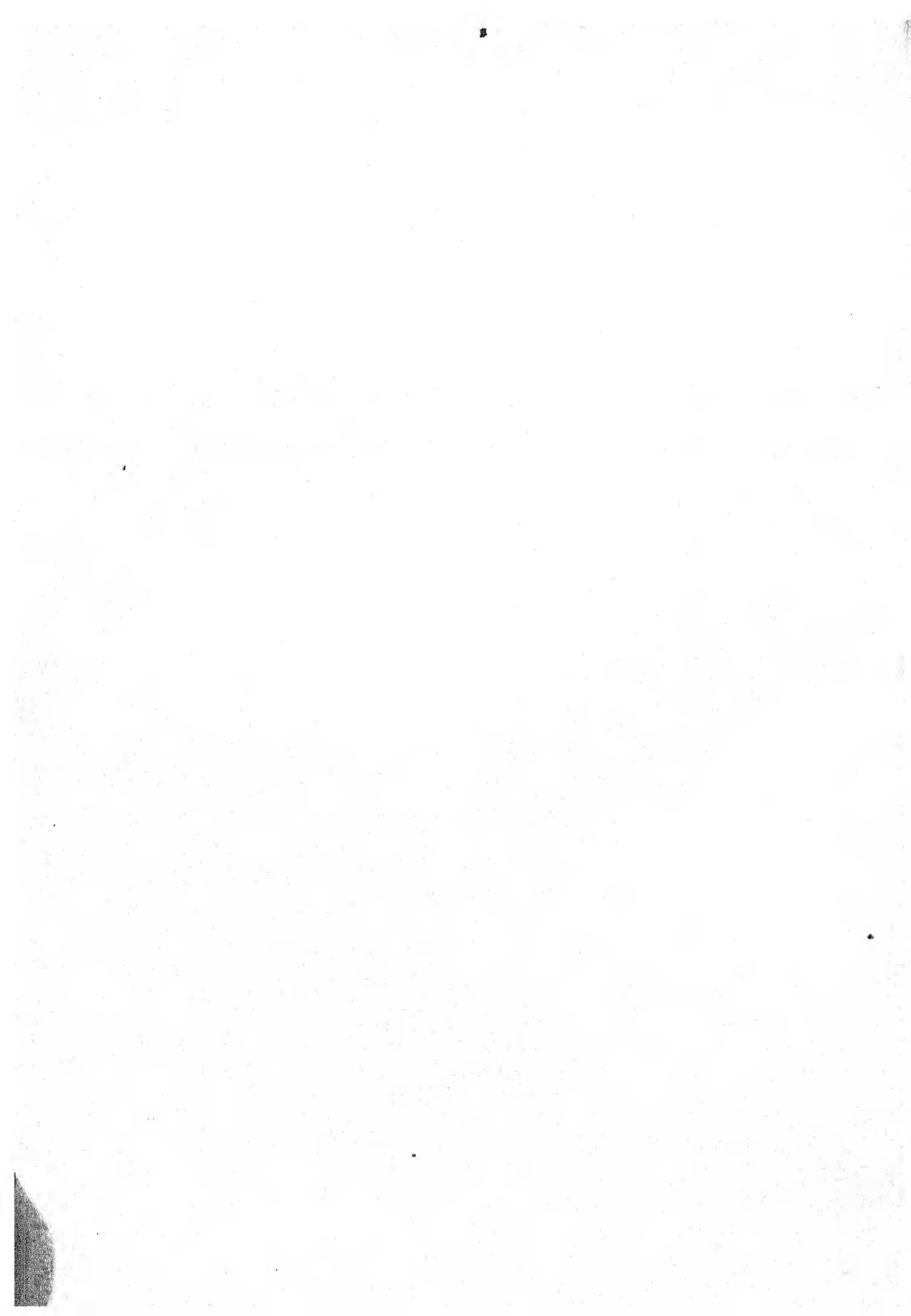
FIG. 12. The same, at the end of the first period of growth;  $P$ , place where the growing point is situated;  $r$ , primary root;  $r^1$ , lateral roots bent down by the contraction of the main root.

FIG. 13. *Lacinaria squarrosa*, subterranean part of a full-grown specimen in summer;  $t$ , tuber;  $r$ , contractile root.





RIMBACH on PERENNIAL HERBS



## CONTRIBUTIONS FROM THE ROCKY MOUNTAIN HERBARIUM. I.

AVEN NELSON.

**Draba Yellowstonensis.**—Annual, with stems of two kinds; the principal stem slender, erect, scape-like, 2–3<sup>dm</sup> high including the raceme, simple or with long-peduncled racemes from the uppermost axil or axils; the one or more accessory stems from the base slenderer and shorter, ascending or erect: leaves mostly basal; root-leaves rosulate, from broadly linear to narrowly elliptic, sub-acute, entire or nearly so, 1–2<sup>cm</sup> long; the few (2–4) stem leaves mostly near the base, narrowly ovate, generally smaller than the root-leaves; pubescence on the leaves finely stellate, on the stems and peduncles sparse, the hairs more or less branching: racemes long in fruit, usually more than half the whole height of the plant: pedicels shorter than the capsules: flowers small; the sepals elliptic, obtuse, about half as long as the petals; the petals cuneate-spatulate, barely emarginate, white, 2–3<sup>mm</sup> long: capsule linear-oblong, tapering slightly to the apex, 10–13<sup>mm</sup> long, finely pubescent; the style very short (less than 0.5<sup>mm</sup> long) but evident; the stigma 2-lobed.

A very distinct species, having its nearest ally in *D. montana* Wats., a plant of more southern range.

Two collections of this were secured in Yellowstone park where it occurs on somewhat shaded, steep, moist slopes: no. 5687 (type), Undine falls, July 6; no. 5922, Yancey's, July 17, 1899.

**Lepidium pubicarpum.**—Annual, with slender vertical tap root; the stem paniculately branched from near the base, in well developed plants the branches similarly branched, only 9–15<sup>cm</sup> high including the racemes, obscurely puberulent: leaves small, glabrous or nearly so, linear or somewhat spatulate, acute, the broader ones remotely cut-toothed: beginning to blossom when very small, the fruiting raceme crowded: pedicels short, hardly so long as the capsule: petals wanting, the sepals purplish:

capsule permanently finely pubescent, from broadly oval to orbicular; stigma sessile in the narrow shallow notch: cotyledons incumbent.

Most nearly allied to *L. apetalum* Willd., from which it differs in being lower and more divaricately branched from near the base, while *L. apetalum* has a stem simple at base and branched above. *L. pubicarpum* is not restricted below the flower cluster, since this is very short and the pedicels become gradually divaricate. Its puberulent capsules separate it at once from *L. apetalum*.

Two collections secured: no. 6235, Nez Perces creek, Yellowstone park, July 30; no. 6793, Dwellé's, Mont., August 31, 1899.

*Arabis densicaulis*.—Biennial or possibly more enduring, the tap root producing several or more often numerous crowded stems from its crown: stems ascending, 3–5<sup>dm</sup> high (including the raceme), leafy only toward the base, simple or some of the larger ones sparingly branched, glabrous or slightly hirsute near the base: root-leaves crowded-rosulate, oblanceolate, short-petioled, 2–3<sup>cm</sup> long, finely stellate-pubescent; stem-leaves rather numerous, glabrous, broadly linear or tapering uniformly from a broadish base to an acute apex, auriculate-clasping, the lobes short: flowers small, the petals white or purplish, linear-spatulate, about 5<sup>mm</sup> long and nearly twice as long as the oblong sepals: the fruiting raceme very long, often two thirds of the whole length; the numerous pods arcuate and widely divaricate or sometimes drooping but not pendulous, 4–5<sup>cm</sup> long, about 2<sup>mm</sup> wide, valves obscurely 1-nerved at base; the pedicels about 1<sup>cm</sup> long: seeds oval, in one row, scarcely winged; the cotyledons oblique, approaching incumbent.

The cotyledons seem to indicate this as a member of the section *SISYMBRINA* (Syn. Fl. 1: 159), but I am unable to find in any of the known species a close ally. It may be recognized easily by its numerous stems crowded on the crowns, and by the numerous, widely divaricate, arcuate pods of the long, naked racemes.

The type specimens were secured on partly wooded, hard, gravelly, north slopes, near Undine falls, Yellowstone park, July 6, 1899, no. 5680.

*Arabis fruticosa*.—Similar in size and habit to the preceding, glabrous throughout except for some fine stellate pubescence at base: the stems even more numerous, with more divergent

bases : crown leaves less crowded, some of them sparsely dentate ; the stem leaves oblong to ovate, the larger ones dentate : fruiting racemes shorter, the pods broader, scarcely arcuate, divaricate-ascending ; the flowers larger as are also the seeds.

No other *Arabis* is known to me that has the habit of this. A single plant sometimes has fifty or more assurgent stems and forms a hemispherical mat several decimeters in diameter. In this respect the preceding species most nearly approaches it. The two were found in the same locality, but they are at once recognized as different.

The type is no. 5681, Undine falls, July 6, 1899.

*Arabis lignipes*.— Short-lived perennial, simple-stemmed or more rarely with two or three stems from the summit of the tap root ; the woody base of the stem persistent, apparently of as many internodes as the plant is years old, leafless, more or less covered with the old petioles ; the internodes variable, usually only 1–3<sup>cm</sup> long, the whole forming a naked woody foot surmounted by the crown of rosulate leaves at the base of the herbaceous part of the stem ; herbaceous stems ultimately 3–5<sup>dm</sup> high, beginning to blossom when quite low, erect, finely stellate-pubescent below, glabrous upward, becoming smooth throughout in age : the rosulate leaves small and crowded, entire, minutely but densely stellate pubescent, narrowly oblanceolate, tapering to a short petiole, 1–2<sup>cm</sup> long ; the stem leaves numerous, sessile, almost linear, tapering to an acute apex from an auricular sagittate base, slightly longer than the rosulate leaves : raceme crowded in anthesis, open in fruit : pedicels sharply deflexed except in the youngest buds, at first minutely pubescent as are also the sepals, 5–7<sup>mm</sup> long : petals purplish or sometimes white, narrowly spatulate, 5–6<sup>mm</sup> long, nearly twice as long as the sepals : pod pendent, straight or curved, smooth, 1-nerved, 6–8<sup>cm</sup> long, about 2<sup>mm</sup> broad : seeds in one row, broadly oval, scarcely wing-margined, about 1<sup>mm</sup> long.

This finds its nearest ally in *A. Holboellii* Hornem., from which its naked woody foot, its invariably simple stems, its smaller entire leaves, and its perennial character separates it.

The following collections of it were secured on dry, sandy or stony bottom lands in Yellowstone park : no. 5503 and 5505, Madison river, June 23, 1900 ; no. 5583, Glen creek, June 30, 1900.

**Arabis pendulocarpa.**—Perennial (probably short-lived), a short, simple or branching, woody caudex surmounting a slender tap root : stems 1–3, only one from each crown, simple, ascending, rather weak, about 2<sup>dm</sup> high, nearly glabrous except at the base which is whitened with simple or branched matted hairs : leaves all entire, crowded on the crowns which are also covered with the remains of those of former years, closely and finely stellate pubescent, narrowly oblong to elliptic, tapering into a short petiole ; the stem-leaves crowded toward the base, linear-oblong, sessile, not auriculate, 5–10<sup>mm</sup> long, usually longer than those of the crowns : flowers few, small, nearly erect at anthesis but the siliques soon pendent : pedicels 6–8<sup>mm</sup> long, glabrous or nearly so : petals white or tinged with purple, about 5<sup>mm</sup> long, distinctly longer than the sparsely hairy sepals : pods 4–6<sup>cm</sup> long, about 2<sup>mm</sup> wide ; the seeds narrowly wing-margined.

The key of the *Synoptical Flora* throws this into close proximity to *A. pulchra* Jones, but it is probably more nearly allied to *A. Holboellii* Hornem.

It occurs among the rocks on exposed or partly wooded hilltops. Secured twice, only in Yellowstone park : no. 5504, Madison river, June 23, 1899 ; no. 5728, Yellowstone river near Junction butte, July 9, 1899.

**Arabis elegans.**—A tall biennial from a vertical tap root, 6–10<sup>dm</sup> high : stem simple and strict (rarely a branch or two from the base), a pubescence of branched hairs below, becoming glabrate upward, leafy up to the inflorescence : leaves crowded on the lower part of the stem but not rosulate at the base, mostly entire, more rarely some of them remotely dentate, finely pubescent or the uppermost almost glabrous ; the lower oblanceolate, petioled, passing into the oblong-linear, sessile, auriculate ones of the middle stem ; the upper gradually smaller, linear, sagittate-clasping : raceme either few- or many-flowered ; flowers rather large, from deep purple to almost white, petals almost twice as long as the pubescent sepals ; buds erect but drooping in anthesis ; pedicels ciliate, in fruit ascending, less than 1<sup>cm</sup> long, bearing divaricate, drooping, or variously curved and twisted pods : pods very slender, 5–8<sup>cm</sup> long, 1–2<sup>mm</sup> wide.

This species bears in its habit a marked resemblance to *A. confinis* Wats., but its narrower pods, which are not beaked, the shorter pedicels, and less glaucous appearance will aid in distinguishing it from its eastern ally.

It is of frequent occurrence in the open woods on moist slopes. No. 5601, Mammoth hot springs, June 30; nos. 5676 and 5680, Undine falls, July 6, 1899, the latter being the type number.

***Arabis divaricarpa*.—**In habit resembling the preceding, possibly sometimes perennial, 4–6<sup>dm</sup> high, glabrous except on the rosulate root-leaves, somewhat glaucous, more or less tinged with purple throughout: root-leaves petioled, slenderly oblanceolate, crowded on the crowns, the pubescence minute and branched; the stem-leaves linear-oblong, sagittate-clasping, 1–4<sup>cm</sup> long: inflorescence glabrous; the flowers purple to white, smaller than in the preceding: pedicels about 5<sup>mm</sup> long: pods uniformly divaricate ascending, straight, 3–5<sup>cm</sup> long, about 3<sup>mm</sup> broad, most of them conspicuously 1-nerved from base to apex.

Like the preceding species, I can compare it only to *A. confinis*, from which it differs in its broader pod, shorter pedicels, mostly entire leaves which on the stem are rather acutely lobed at the base. The seeds also are quite different; in *A. confinis* small, narrowly oblong; in *A. divaricarpa* rather large and nearly oval.

It was secured in two localities on the open, sandy hillsides overlooking Yellowstone lake, August 1899, nos. 6352 and 6622.

***Viola Thorii*.—**Root mostly simple, semi-fleshy, rather large for the plant: stems several, short, about 15<sup>mm</sup> high: leaves on petioles 2–3<sup>cm</sup> long, broadly ovate, truncate at base or abruptly narrowed into the petiole, very coarsely and bluntly dentate, sparsely puberulent below or entirely glabrous, 12–20<sup>mm</sup> long: peduncles usually surpassing the leaves, very sparsely puberulent, 2–4<sup>cm</sup> long: sepals linear-lanceolate, glabrous, 4<sup>mm</sup> long or less: petals about 1<sup>cm</sup> long, glabrous, yellow, the two upper reddish-brown on the back.

*Viola atriplicifolia* Greene, which I have not seen, is evidently a close ally, but the species now proposed lacks the cinerous puberulence of that, and has nearly entire leaves in contrast with the hastate or lobed leaves of the other, which also has thickened or flattened petioles.

The type specimens, no. 5816, were secured on the moist, open slopes near the summit of the Thunderer, Yellowstone park, July 13, 1899. I has

also been collected by Dr. Blankinship in Montana and in Yellowstone park, but I am unable to cite his numbers.

**Epilobium Wyomingense.**—Perennial, spreading by filiform remotely scaly subterranean shoots which end in ovoid winter bulblets with few fleshy scales: stems slender, 2–4<sup>dm</sup> high, strictly erect, mostly simple, more rarely with slender, erect branches from the axils of the opposite leaves; wholly glabrous below, towards the summit of stems and branches (if any) an obscure puberulence: leaves thin and glabrous, linear, tapering from the middle to both ends, sub-acute, from 3–5<sup>cm</sup> long (rarely even 8<sup>cm</sup> long), 2–5<sup>mm</sup> broad, the uppermost not noticeably reduced, midrib evident, the lateral veins obscure, plane, or the margin barely revolute, opposite except the floral, the few (4–7) pairs nearly equidistant, often shorter than the internodes; those of the branches similar: flowers several, erect, small; the calyx cleft nearly to the base; the petals white, ovate, deeply triangular-notched at apex, 3–4<sup>mm</sup> long, a little longer than the sepals: capsules linear, 4–7<sup>cm</sup> long, minutely cinereous-puberulent; the pedicels variable, from much shorter to even exceeding the capsule: stigma oblong or short-clavate, barely notched at apex: seeds numerous, fusiform, smooth, scarcely beaked; the coma white, persistent.

This species is not very closely allied with any of the species known to me, though in a few respects it suggests *E. Oregonense gracillimum* and *E. glaberrimum*. In the more essential characters it seems to be allied to *E. palustre*, from which its longer smooth leaves and smooth stems, and its smooth almost beakless seed separate it.

It occurs in dense patches, on the grassy, boggy banks of spring streamlets. Yellowstone park, no. 5902, Yancey's, July 16; no. 6428, near Snake river, August 12, 1899.

**Cryptanthe multicaulis.**—Several to many-stemmed from the crown of the vertical tap root; the stems rather slender, ciliate-hirsute, erect from a mostly short-decumbent base, sparingly paniculate-branched above, 15–20<sup>cm</sup> high: leaves rather numerous, broadly linear, 2–3<sup>cm</sup> long, the unequal, whitish, hispid hairs with pustulate bases: spikes slender, moderately dense even in fruit: sepals setose-hispid, the stouter setae yellowish, the



midrib not evidently thickened, very narrowly lanceolate-acuminate, about 5<sup>mm</sup> long: nutlets obscurely roughened under a lens, ovate with sub-acute apex, less than 2<sup>mm</sup> long, the narrow groove forked at base but without conspicuous open areola, similar, usually only three maturing.

This species is allied to *C. Pattersoni* and to *C. ramulosissima*. In habit it is intermediate between them, but in floral and fruit characters it differs essentially from both.

The type number is 6440, from Snake river, Yellowstone park, August 13, 1899.

**CRYPTANTHE AFFINIS flexuosa.**—Evidently allied to *C. affinis*, but a larger plant, 3–5<sup>dm</sup> high: stems loosely branched from near the base upward, the branches long and flexuous: leaves narrowly oblong, 2–3<sup>cm</sup> long: fruiting spike long and open: sepals lanceolate-acuminate, densely hispid at base, the tips mostly open and spreading, 6–8<sup>mm</sup> long: nutlets mottled, smooth and shining, ovate-acute, about 2<sup>mm</sup> long, the ventral groove nearly closed, forked at the base but without areola.

This may prove worthy of specific rank, but until further material is secured it may best stand as a variety. The species, I think, occurs further toward the west and northwest only.

Secured in Jackson's hole, near Jackson's lake, August 17, 1899.

**Mertensia amoena.**—Root somewhat woody, more or less branched, surmounted by a branched caudex: branches of the caudex few to several (3–8), crowded, erect, closely covered with dead leaf-bases: stems one or more from each crown, simple, ascending, 1–2<sup>dm</sup> high, pubescent with short spreading or crisped hairs: crown-leaves oblong-lanceolate, sub-acute, glabrous below, softly hispid-pubescent above and on the margins, 4–7<sup>cm</sup> long, about one fourth as broad, on slender petioles which are usually longer than the blade: stem leaves rather crowded, broadly linear or narrowly oblanceolate, sessile, 3–6<sup>cm</sup> long, pubescence similar to that of the root-leaves; inflorescence crowded; calyx-lobes lanceolate, sparsely ciliate-hirsute, about half as long as the tube of the corolla; corolla about 15<sup>mm</sup> long, the tube scarcely longer than the campanulate limb, the lobes of the limb about half its length, crests inconspicuous; filaments

broad as the anthers, inserted in the throat, the free portion about as long as the anther; the style nearly as long as the corolla.

This is probably a part of the *M. lanceolata* (Pursh) DC. of Gray in Proc. Am. Acad. 10: 53, and of the Syn. Fl. 2: 201, though it is very distinct from what Pursh and De Candolle understood by that species. The original seems to have been that glaucous, glabrous (at most slightly scabrous) plant which we know from the eastern slopes and foothills of the Rocky mountains, and which extends eastward toward the Missouri in the hill regions. That has rather thick fleshy leaves and has fewer stems. I have for years been familiar with it in southern and eastern Wyoming. During the season of 1899 Montana and northwestern Wyoming, where the species now proposed is common, came under my observation. It did not occur to me that anyone could possibly have called it *M. lanceolata*, so different are they in the field. *M. amoena* may be recognized by its caespitose habit, its hirsute (almost cinereous and never glaucous) leaves and stems, and the more crowded inflorescence, which in young plants reminds one of *M. oblongifolia*. It has the habit and leafiness of *M. foliosa*, and is more clearly distinct from *M. lanceolata* than *M. Fendleri* is from that species.

Collected at Monida, Mont., June 15, no. 5413; Glen creek, Yellowstone park, June 29, 1899, no. 5556; at both of which places it was abundant.

**Solidago dilatata.**—Perennial, from a woody root surmounted by a branched caudex bearing a few surculiferous branches which terminate in a fascicle of leaves: stems single from the crowns, simple, or branched above, rather stout, somewhat striate, glabrous, 4–6<sup>dm</sup> high: leaves glabrous, conspicuously reticulate-veiny below, minutely scabro-ciliate on the margins; basal leaves oblong-spatulate to elliptic, tapering into a broad margined petiole which is sometimes as long as the blade, either closely or remotely serrate, mostly obtuse at apex, 8–15<sup>cm</sup> long; the stem leaves numerous, sessile, mostly small (2–3<sup>cm</sup>): inflorescence nearly glabrous (some ciliate straggling hairs), paniculately corymbose, either compact or quite open, the lower pedunculate branches leafy bracteate: heads numerous, slender-pedicelled; the disk about 6<sup>mm</sup> high; involucral bracts in about three rows, the shorter outer ones very few, the two inner rows sub-equal, minutely ciliate on the margins, linear, most of them obtusish and slightly dilated upwards: rays 8–10, conspicuous; akenes short and lightly pubescent.

This is to be compared with *S. multiradiata scopulorum*, but it is a much larger plant, with larger root-leaves and larger more open inflorescence. In being practically glabrous it also differs from that, and the upwardly dilated bracts especially distinguish it.

It was abundant in loose gravelly soil in the open woods in the southern part of Yellowstone park, no. 6586, August 21, 1899.

***Machaeranthera superba*.**—Probably only biennial, very numerously branched from the crown of the slender tap root: stems decumbent at base and widely spreading, 8–15<sup>cm</sup> long, each simple below, but paniculately corymbose as to the inflorescence, their purplish hue masked by a minute cinereous puberulence; root-leaves (mostly wanting at flowering time) oblong-lanceolate, with minute spine-tipped teeth, cuspidate-obtuse, tapering into a slender petiole somewhat shorter than the blade, whole length 4–6<sup>cm</sup>: stem leaves rather numerous, broadly linear to narrowly oblanceolate, entire or remotely denticulate, the teeth and apex cusped as in the root-leaves, minutely and softly subcinereous (scarcely canescent), 3–5<sup>cm</sup> long, smaller in the inflorescence: heads moderately large, disk about 1<sup>cm</sup> high, nearly as broad; bracts of the involucre oblong, acute, tips mostly erect, decidedly tinged with purple which is only slightly obscured by the thin puberulence, very rarely a few gland-tipped hairs on the margins: rays 12 (more or fewer), a deep blue (possibly sometimes varying to a purple), length mostly less than 1<sup>cm</sup>.

Of this species, which was submitted with a number of others, Dr. Greene writes as follows: "A subalpine looking, too showy form of *M. canescens*. But it cannot be referred to *M. subalpina*, however much it looks like it at first glance." Since that was written I have given much study to both of the above species, of which I have typical specimens (the type number of the latter). I am satisfied that the species now proposed is amply distinct from both.

*M. canescens* as understood (evidently) by Pursh, Nuttall, Gray, and Greene is an essentially erect plant even though branched from the base up, some of its leaves are distinctly serrate or toothed, the bracts are evidently green-tipped, the pubescence is canescent rather than cinereous. *M. superba* is more nearly allied to *M. subalpina*. This it resembles in the relatively few (as compared with *M. canescens*) showy heads and in the character of the pubescence. It is distinguished from it by the almost entire absence of viscid

or glandular hairs, and from both by its less erect habit and by its broad, colored involucre bracts.

It occurred in the greatest abundance in one locality only, an open, sandy hillside near Yellowstone lake. The depressed, mat-like plants, with their relatively large, showy heads, were singularly attractive and invited the closest attention. The type number is 6337, from the Thumb, August 6, 1899.

**Erigeron Yellowstonensis.**—Biennial, or probably many of the plants more enduring, with a strong vertical tap root: generally only one stem from the enlarged crown (more rarely 2–5), simple, stout, striate, erect, paniculately branched as to the inflorescence, 3–6<sup>dm</sup> high, purplish, glabrate, the whitish hairs very straggling, obscurely granular (scarcely glutinous): leaves numerous, pubescence nearly wanting, similar to that of the stem; crown leaves oblanceolate, petioles 3–6<sup>cm</sup> long; lower stem leaves similar but with short winged petioles; upper leaves sessile, narrowly lanceolate, not much reduced, the short branches of the panicle from their axils; bracts small, linear: heads numerous, on rather slender peduncles; involucre bracts dark green, in two rows, subequal, very narrow, acuminate, shorter than the 1<sup>cm</sup> high disk: flowers very numerous; rays filiform, purplish, only moderately numerous, largely concealed by copious pappus: akenes linear, appearing glabrous but sparsely pubescent under the microscope, less than 2<sup>mm</sup> long; the soft, dirty-white pappus nearly three times as long.

The *E. Droebachensis* Mueller (*E. acris Droebachensis* (Muel.) Blytt.) of Europe is unknown to me, but I cannot read the description of that into the plant now proposed as a species. Neither does it seem probable that any of the other usually accepted synonyms of *E. Droebachensis* represent this plant, for they also refer to European or arctic forms, except the *E. glabratus* of Hooker's *Flora*. The latter seems to have spatulate root-leaves, the cauline ones almost linear; a racemiform inflorescence, with very long lower peduncles, making an approach to a corymb; the pappus of a more yellowish hue.

This plant was found in abundance near Yellowstone lake, in the open pine woods, in loose sandy soil: nos. 6348 and 6615, the Thumb, August 1899.

**ERIGERON MULTIFIDUS incertus.**—Caudex densely cespitose; its branches comparatively long and woody, roughened with the

old leaf-bases: stems usually two or more from each crown, rather slender, curved ascending or erect, 12–18<sup>cm</sup> long, sparsely ciliate and obscurely granulo-puberulent, monocephalous: leaves crowded on the crowns, green and appearing glabrate but ciliate on the petioles and often on the blades, obscurely granulo-pubescent, simply three-parted, or more often each segment again three-parted; ultimate segments linear-oblong, 4–6<sup>mm</sup> long; petioles slender, 2–3<sup>cm</sup> long; stem leaves few (2–4), bract-like, mostly linear, entire, the lower occasionally trifid: heads 8–12<sup>mm</sup> high, rays wanting (apparently sometimes a few filiform ones); disk flowers very numerous; involucre ciliate-pubescent and granulo-glandular under the longer hairs, bracts linear-acuminate, equaling the disk: mature akenes flattened, narrowly obovate, finely pubescent, about 2<sup>mm</sup> long; the soft pappus about twice as long as the akene.

This variety holds about the same relation to *E. multifidus* Rydb. (Fl. Mont. & Y. N. P. 402) as the variety *glabratus* does to the same species. The two varieties resemble each other in habit and leaf outline. They are both glabrous in appearance, but only the one is wholly glabrous and the other is eradiate. The variety *incertus* differs from *E. multifidus discoidens* in its larger size, in being more glabrate, and in its cespitose caudex.

Nos. 5538 and 6066 from Yellowstone park belong here, as does also no. 2343 from Dome lake in the Big Horn mountains, 1896. It occurs on dry stony hills and is fairly abundant.

**ARNICA CHAMISSONIS longinodosa.**—Stems single from horizontal rootstocks from which spring numerous, thick, fibrous roots, erect, 4–8<sup>dm</sup> high, the internodes long, usually 10–15<sup>cm</sup>, much exceeding the leaves or rarely only equaling them: pubescence of two kinds, a short, dense, glandular puberulence (especially on the peduncle), and some scattering white crisped hairs (especially on the involucre): leaves 3–5 pairs, denticulate; the lowest pair oblong-ob lanceolate, 6–10<sup>cm</sup> long, on slender petioles often as long, disappearing early in the season or, at least, rarely collected; the next lowest mostly obovate or narrower, tapering into a short margined petiole, variable in size, either shorter or longer than the lowest pair; the upper pairs sessile by a broad base, very variable in size and shape, from

ovate to oblong, 5–15<sup>cm</sup> long: heads 1–3, mostly single and then long-peduncled; if more, then from the axils of the uppermost leaves which are often much reduced, occasionally only one of the leaves and lateral peduncles developing; lateral peduncles equaling or even exceeding the terminal, 1<sup>dm</sup> (more or less) in length: heads large (if more than one somewhat reduced), disk 15–20<sup>mm</sup> high, 20–30<sup>mm</sup> broad; rays about twelve, 20–30<sup>mm</sup> long, 6–8<sup>mm</sup> broad; involucre bracts 14–20, lanceolate, acute, shorter than the disk: akenes striate, nearly linear, but tapering to the base, obscurely short-hispid on the angles, about 5<sup>mm</sup> long, equaling the sordid, sub-plumose pappus.

I have described this in detail, for I believe that it will ultimately be shown that this is a good species rather than a variety. The *A. Chamissonis* Less., from Unalashka, seems to be a much more pubescent plant, with narrower leaves and more pubescent akenes, the internodes, as compared with the leaves, relatively shorter.

The following numbers represent some of the collections of this species in Wyoming: 1702, 1785, 3571, and 6379, the last from Yellowstone park, August 1899. M. E. Jones no. 5883, from Utah, is this species.

**Arnica Columbiana.**—Perennial, 4–8<sup>dm</sup> high: stems rather stout, erect, striate, simple below, paniculately corymbose above, with some lanate white pubescence: root-leaves not known; stem leaves several (six or more pairs not counting the rameal), the lower apparently petioled, the middle and upper ample, sessile, clasping, entire, rather conspicuously nerved, oblong, sub-acute, finely pubescent below, obscurely so above, 8–14<sup>cm</sup> long, 3–5<sup>cm</sup> broad; rameal leaves and bracts ovate, the smaller ones acuminate: inflorescence an ample paniced cymose corymb of 15–30 or more rather unequal heads: involucre bracts 20 (more or less), oblong, sub-acute, shorter than the disk: rays 12 or less, 10–14<sup>mm</sup> long, 3–4<sup>mm</sup> broad: akenes small, 5-nerved, linear-subcylindric, tapering to a slender base, only about 3<sup>mm</sup> long, shorter than the sordid pappus.

Of this unusually distinct species I have seen but two specimens, both of which are in the herbarium of the Montana State College.

The type is Mrs. J. J. Kennedy's no. 24, Columbia falls, Mont., 1894. Mr. R. S. Williams' no. 1049, from the same locality, June 14, 1894, is the

same as to the larger specimen (on the sheet before me). Both were distributed as *A. amplexicaulis* Nutt., to which they bear but little resemblance. Mrs. Kennedy's specimens are included by Dr. Rydberg in his *A. amplexifolia* (Nutt.) of his *Flora of Montana*.

**Arnica ocreata.**—From slender horizontal rootstocks: stems slender, 3–4<sup>dm</sup> high, erect, very leafy, nearly glabrous, more or less finely granular-glandular, occasionally some straggling woolly hairs: leaves 6–10 pairs, ascending or erect, from broadly to narrowly lanceolate, entire, sub-acute, gradually smaller upward and becoming bract-like, all but the uppermost much exceeding the short internodes (even overlapping two or three of the internodes), the lowest petioled, the uppermost sessile: petioles slender, dilated at the base and connate, the pair forming an ocrea or sheath which in the lowest leaves is 2–3<sup>cm</sup> long, the sheath and petioles gradually shorter upward to about the middle of the stem where both become wholly absent: heads one to several; if three, corymbose, the two from the axils of opposite bracts; if more than three, mostly somewhat racemose from the axils of alternate bracts; terminal head largest, disk 10–12<sup>mm</sup> high, exceeding the oblong, obtusish bracts, about 15<sup>mm</sup> broad; rays about 1<sup>cm</sup> long; lateral heads somewhat reduced: pedicels variable, rather slender, very lightly woolly-pubescent: akenes nearly glabrous, lightly striate, somewhat flattened, narrow, tapering to the base, nearly equaling the soft pappus.

This is in part, so far as specimens in the collections go, *A. foliosa*. It is not the *A. foliosa* of Nuttall, which is probably a rarer plant, though collections of that are not infrequent. That, as may be seen by the original description and as may be gathered from Hooker's *Flora* and from Torr y and Gray's *Flora N. A.* (where it was compared with *A. montana* and *A. Chamissonis*) is a very different plant, and nearly allied to *A. Chamissonis*. *A. foliosa* has the stout, strict habit of that, is tomentose pubescent, somewhat equally leafy throughout, the leaves callous-denticulate, evidently nerved (about five), the heads congested-corymbose and the akenes hirsute. *A. ocreata* may be recognized at once by the characteristic disposition of its entire, rather narrowly lanceolate leaves. Owing to their diminution in size upward and the comparatively long petioles of the lower (some of them disappearing early) the strict leaves have the appearance of being crowded near the middle, while the base and summit of the stem are semi-nude.

This species occurs on wet bottom lands in the edges of copses of under-shrub. Typical collections of it are nos. 766 and 1195, Wind river, Wyoming, Aug. 1894, by *Nelson*; no. 5224, Shoshone lake, Aug. 1897, by *Rydberg & Bessey*; no. 6403, Snake river, Aug. 1899, by *Nelson & Nelson*; Silverton, Colo., July 1898, by *C. S. Crandall* (distributed as *A. longifolia*).

***Arnica polycephala*.**—Nearly allied to and much resembling *A. longifolia* Eaton, but larger than that species, forming larger and denser clumps, the stems very numerous: leaves lanceolate, less acuminate than in *A. longifolia*, glabrous or obscurely granulo-glutinous: heads moderately large, very numerous (20–50) on each stem which is paniculately branched above.

This might possibly be considered only a variety, but it is at once distinguished from its ally by the absence of the puberulence of that species, by the less acuminate leaves, and the several times more numerous heads.

It was found growing in great masses among the rocks of the slides, of the steep cliffs overlooking Snake river near the southern boundary of Yellowstone park. The type is no. 6422, Aug. 12, 1899.

***Arnica exigua*.**—Low, 1–2<sup>dm</sup> high, sub-cinereous hirsute-pubescent throughout, with an obscure granular glutinosity beneath: stem erect, corymbosely paniculate-branched from the base upward; the branches ascending, often a pair from each node, each bearing one or two heads: leaves mostly lanceolate, acute; the two or three pairs on the main stem 5–8<sup>cm</sup> long, all sessile; those on the branches similar but smaller: heads of medium size, involucre bracts oblong, sub-acute, nearly equaling the disk which is 10–15<sup>mm</sup> high; rays in well developed heads twelve or more; akenes very narrow and tapering to the base, nearly glabrous.

This is a species of unusual habit. It has the appearance of a plant which has put out new shoots after being browsed off and stunted by some animal. An examination, both in the field and of the specimens collected, shows that this is not the case, and that this dwarfed plant is perfectly normal. It is an ally of *A. foliosa* Nutt., of which I at first suspected it being a deformed state. The habitat of the two is different. *A. foliosa* is found on the fertile soils of wet bottom lands.

The specimens of *A. exigua* were secured on the higher, dry sandy bluffs and ridges overlooking Yellowstone lake. The type is no. 6940, Aug. 24, 1899.



*Arnica caespitosa*.—Low (1–2<sup>dm</sup>), matted, sometimes forming beds several decimeters across, with moderately large woody horizontal rootstocks from which spring numerous thick fibrous roots, the whole forming a dense turf: the numerous stems erect, sparsely short-lanate as is also the base of the involucre: leaves nearly glabrous or sparsely ciliate-woolly, three or four pairs on the stems and some fascicled ones on the sterile crowns; crown leaves mostly oblanceolate, petioled, 4–7<sup>cm</sup> long (including the petiole); basal stem leaves very small (often wanting), obovate as also the next larger pair; upper leaves lanceolate: heads one to five, large for the size of the plant, mostly three (a terminal one and a pair from the uppermost axils); pedicels moderately stout, 2–5<sup>cm</sup> long: involucre turbinate; bracts linear-oblong, sub-acute, almost equaling the disk; rays 8–10, ascending, rather broad: akenes linear, white-pubescent, about 5<sup>mm</sup> long, equaling the white, glistening pappus.

An excellent species of nearly alpine stations; occurring in patches on nearly naked, rocky slopes. It may be recognized by its caespitose habit and dense root-system, its turbinate head (the rays are ascending also), and the white-pubescent akenes.

Collections of it are no. 5785, Druid peak, Yellowstone park, July 12; no. 6717, Teton mountains, Aug. 16, 1899.

LARAMIE, WYO.

## BRIEFER ARTICLES.

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### PHOTOGRAPHY IN BOTANY AND IN HORTICULTURE.

(WITH TWO FIGURES)

ALMOST every working scientist knows something about photography, and probably no one will deny that a camera is one of the indispensable equipments in every well-regulated botanical or horticultural laboratory. Yet a casual acquaintance among various horticulturists and botanists, and the repeated publication of unnecessarily inadequate (not to say atrociously bad) photographs, lead us to believe that the use of the camera in horticultural and botanical work will bear some discussion. We are convinced that the value of the camera as a piece of scientific apparatus is not generally appreciated. We believe that the camera is not used as often as it ought to be, and we believe yet more strongly that the photographic methods with which many science workers content themselves are not really creditable. We believe that as a help in daily work, the camera ranks next to the microscope for the botanist, and far above the microscope for the horticulturist. Yet while every laboratory has a row of books on microtechnique, and while every botanist and most horticulturists work hard to perfect themselves in microscopical methods, a book on photography is a rarity, and men are satisfied to blunder along with almost any sort of a camera, and with plates and developers of which they know practically nothing. Some even are willing to "press the button" and let some company in another state "do the rest."

In the matter of photographing plants, fruits, flowers, and similar objects,<sup>\*</sup> we may perhaps offer a few observations as the result of experiments covering several years.

The particular piece of apparatus required for this sort of work is some support which will hold the camera approximately in a vertical position, and will provide a transparent horizontal shelf some distance from the floor. The objects are laid on the latter and the camera is focused down upon them.

<sup>\*</sup>"Photographing flowers and trees," *The Photo-Miniature*, No. 13, April 1900.

Any sort of construction which provides for the vertical camera and the horizontal shelf will answer, and several different forms are actually in use. *Fig. 1* is sketched from a photograph of the apparatus in use at the Vermont Experiment Station. *Fig. 2* shows a more elaborate form, designed by Mr. McFarland and used at the Mt. Pleasant Printery. The construction will be obvious in either case, and may be modified to suit individual needs. Lightness, rigidity, and portability are the chief requirements in an apparatus for field use; while in the studio the permissible increase of weight makes a somewhat more convenient construction available. *Fig. 2* is especially a studio camera-stand, adaptable for both vertical and horizontal work, making lantern slides, enlargements, etc.

The advantages of such an apparatus are: (1) It does away with nearly all trouble in arranging the subject. Simply lay a plant, some flowers, specimens of fruit, or other objects on the glass shelf, and they stay where they are put. In the case of fruits, which will not always lie where wanted, one should have at hand a supply of rubber hose-washers, *i. e.*, rubber rings 3-4<sup>cm</sup> in diameter. Set the fruit into the ring, and it may be moved anywhere and put in almost any position. (2) It allows one to arrange any background desired. Sheets of cardboard in white, black, and neutral tints may be successively tried behind the subject and the best one chosen. (3) It does away with shadows which are almost inevitable and frequently disastrous in any other method of plant photography. (4) It facilitates considerably the work of making exact size photographs of objects. For all sorts of floral and fruit photography it is much the best way to have all photographs (with a few necessary exceptions) made exact size. This is especially desirable when one begins to have a collection of negatives numbering into the thousands.

For comfortable plant, flower, vegetable, or fruit photography in full natural size, a camera with considerable bellows extension is essential. To obtain a natural size image the lens must be equidistant between the object and the ground glass of the camera at twice its focal length. Thus, if for a plate of  $6\frac{1}{2} \times 8\frac{1}{2}$  inches a lens of  $7\frac{1}{2}$  inches

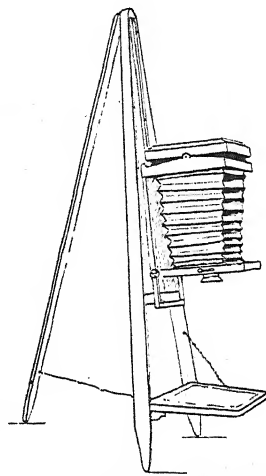


FIG. 1.

focal length be used, sufficient bellows must be at hand to permit the ground glass to be fixed at 15 inches from the objective. The modern "long-focus" cameras provide very fully for this need and others which will appear upon trial. The best work will be possible if a lens is chosen of rather short focal length, one technically known as a "wide-

angle" lens. Not only is the range of the camera thus increased, but the sharp focus over a considerable depth which is absolutely requisite in morphological work is far more easily obtained.

In focusing, it will be found a decided advantage to select a spot in the object or composition upon the glass shelf a little above its vertical center, and there to affix temporarily a white card or paper with fine lines. An ordinary visiting card is excellent. When this is focused upon, and the lens then "stopped down," the whole object will be sufficiently sharp. We urge extreme care and cleanliness in all photographic operations. In working full size, dust is a great

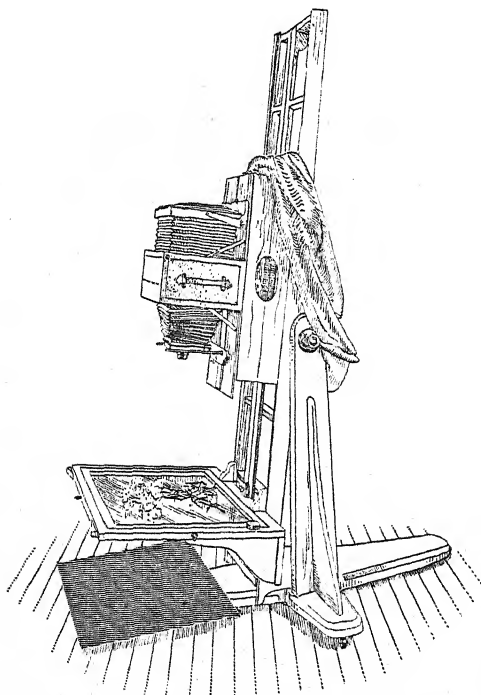


FIG. 2.

enemy; every grain is willing to be photographed.

A skylight is most undesirable for vertical photography, because of the reflections upon the glass platform. Indeed, the worker will find it almost essential to guard against reflections from the skylight, the ceiling of a light room, and particularly from the polished camera front and lens, by preparing a wire frame or hood, covered with black velvet or the like, and extending about the camera so as to cut off the immediate top light.—F. A. WAUGH and J. HORACE MCFARLAND.

## CURRENT LITERATURE.

### MINOR NOTICES.

T. ITO AND J. MATSUMURA<sup>1</sup> have begun the publication of a catalogue of the flora of the Lûchû Islands, the archipelago between Japan and Formosa. The present work, written in English, is intended to be a preliminary contribution to the knowledge of the flora of these islands, containing merely the bibliography, synonymy, and local and general distribution of the plants, with occasional descriptions and critical notes. The Leguminosae, including about 125 species, are specially worked out by Professor Matsumura, a number of new species being described. The flora is one of exceptional interest, and such a compact and complete presentation of it will be welcome to botanists. —J. M. C.

O. WARBURG has begun the publication of the flora of the monsoon regions of southern and eastern Asia, selecting as his title *Monsunia*.<sup>2</sup> The typography is sumptuous, and the plates are of the highest quality. A brief itinerary shows a most extensive personal experience throughout the vast region indicated by the title. The Fungi are by P. Hennings, *Phaeolomacium* (Agaricaceæ), *Nymanomyces* (Hysteriaceæ), *Phaeorhizisma* (Phacidiaceæ), *Cerocorticium* and *Discocyphella* (both Thelephoraceæ), *Pseudotrype* (Hypocreaceæ), *Pseudothia* (Melanommaceæ), *Schizacrosporum* (Acrospereaceæ), *Janseella* (Stictaceæ), and *Phaeomacropus* (Pezizaceæ) being new genera from Java. The Algæ by F. Heydrich, and the Hepaticæ by V. Schiffner have been for the most part already published elsewhere. The Musci are by V. F. Brotherus (*Warburgiella* being a new genus), and the Filicineæ by H. Christ, the latter group naturally having a very extensive representation. The other pteridophyte groups are presented by Warburg, and abound in new species and critical notes. The genus *Selaginella* is especially considered, being represented by 184 species, forty-seven of which are ascribed to the author, and its geographical distribution is discussed. The gymnosperms, which close the volume, are also by Warburg, and this part is full of interest, containing as it does descriptions, fine illustrations, and distribution of many of the little known and more critical forms. —J. M. C.

<sup>1</sup> Tentamen Florae Lutchuensis. Sectio I. Plantae Dicotyledonæ Polypetalæ. Separate from Jour. Sci. Coll. Imperial Univ., Tokyo, 12: 263-541. 1899.

<sup>2</sup> Monsunia, Beiträge zur Kenntniss der Vegetation des süd- und ostasiatischen Monsungebietes, Vol. I, pp. 207. pls. 1-11. 1900. Leipzig: Wilhelm Englemann. M 40.

## NOTES FOR STUDENTS.

THE "PROTEID VACUOLES" of gymnosperms have recently been reinvestigated by Arnoldi.<sup>3</sup> These structures were described by Hofmeister nearly fifty years ago as "Keimbläschen" (germinal vesicles) and he makes the statement that the archegonium of the vascular cryptogams differs from that of the gymnosperms in having only one nucleus (Keimbläschen) while that of the gymnosperms has many. Schacht, who wrote at about the same time, regarded the structures as cell-sap vacuoles, while Strasburger, at a considerably later period, regarded them as proteid vacuoles.

Goroschankin (1883), who made some study of their development, found that they differ from the sap vacuoles, and make their appearance before the cutting off of the ventral canal cell, disappearing during the formation of the embryo.

The present writer, with the aid of modern technique, has attacked the old problem of the origin and development of the bodies and finds that they are genuine nuclei. The nuclei of the cells of the tapetal layer immediately surrounding the oosphere put out amoeboid processes which penetrate the cell wall, and soon the whole nucleus passes through the opening and into the cytoplasm of the egg, where they become more or less modified so that while they bear a striking resemblance to nuclei they could not be positively identified as such except by tracing them back to their origin. Nuclei were also observed to pass from the next layer into the cells of the tapetal layer. The paper is only a preliminary one, but the stages figured are strong evidence in favor of the author's view. These bodies serve only for the nutrition of the embryo and take no part morphologically in its development.—CHAS. J. CHAMBERLAIN.

DUNCAN S. JOHNSON has been investigating *Saururus cernuus*<sup>4</sup> in reference to the claim made by Engler and others that it is a primitive dicotyledonous type. He finds in the developing ovule a one-celled archesporium; a "tapetal" cell is cut off, which gives rise to but few cells; the megaspore mother cell organizes a row of three, the lowest of which becomes the functioning megaspore; the ante-fertilization development of the sac structures is as usual; the antipodal cells are evanescent. After the ante-fertilization stage is reached the sac grows rapidly, especially in length, and finally broadens below, remaining narrow at the top, thus appearing as a long-necked flask, with the primary endosperm nucleus lying at the base of the neck. At the first division of this nucleus one daughter nucleus remains in

<sup>3</sup> ARNOLDI, W.: Beiträge zur Morphologie der Gymnospermen. IV. Was sind die "Keimbläschen" oder "Hofmeister's Körperchen" in der Eizelle der Abietineen? Flora 87: 194-204. pl. 6, 7. 1900.

<sup>4</sup> On the development of *Saururus cernuus* L. Bull. Torr. Bot. Club 27: 365-372. pl. 23. 1900.

the neck, the other moves down into the body of the flask, and a wall is formed across the base of the neck, giving rise to two endosperm chambers. The upper endosperm nucleus divides and forms a compact tissue in the upper chamber; while the other enlarges but never divides, the lower and larger endosperm chamber apparently being related to the adjacent perisperm as an absorbing organ. In the upper chamber the embryo is organized, and the endosperm about it encroaches upon all the nucellar tissue adjacent to it.

The germination of the seed is also very peculiar, the endosperm emerging first, and retaining hold of the cotyledons and supplying nutrition after the cotyledon tips have carried the old seed well up into the air. The author concludes that there is no evidence that *Saururus* is more primitive in character than many other dicotyledons.—J. M. C.

FR. SCHAIBLE has devised an apparatus for research on the effect of diminished air pressure on plant growth,<sup>5</sup> in which the plants can be cultivated for a considerable time without being in a stagnant atmosphere and without being subjected to repeated evacuation of the culture chamber. The essential feature is the use of a tubulated bell jar to which air is admitted through a capillary tube, a water pump in continuous operation serving to exhaust the chamber more rapidly than the air can enter through the capillary passage.

He finds, in agreement with Wieler and Jaccard, that ordinary growth is accelerated, especially that of the leaves, while germination is retarded and is of lower percentage. Investigation of the question whether these results are due to diminished pressure, as such, or to diminished partial pressure of oxygen, leads Schaible to conclude that the retardation of germination is due to the diminished O pressure. The acceleration of growth, however, is not due to this cause (which really tends to retard growth slightly), nor to lessened CO<sub>2</sub> pressure, nor to the altered light, heat, or moisture. The only possible explanation seems to be that the plants absorb water more rapidly under the lower pressure (isolated cylinders of live pith do so), and consequently the cells reach their definitive size more quickly, thus attaining earlier the stage of division. The copious guttation observed both by Döbereiner and Schaible also accords with this explanation.—C. R. B.

ITEMS OF TAXONOMIC INTEREST are as follows: A. ENGLER (Bot. Jahrb, 28: 385-510. 1900) has completed his account of the flora of the Lake Nyassa region, describing numerous new species. *Neogoetzea* Pax (Euphorbiaceæ), *Linnaeopsis* Engler (Gesneriaceæ), *Megalopus* K. Schumann (Rubiaceæ), and *Ageratina* C. Hoffmann (Compositæ), are the new genera.—HENRY DEANE and J. H. MAIDEN are publishing in Proc. Linn. Soc. of South Wales a series

<sup>5</sup>Fünfstück's Beitr. z. wiss. Bot. 4: 93-148. 1900. See also Bot. Centralbl. 82: 52. 1900.

of papers entitled "Observations on the eucalypts of New South Wales." The last one issued (Proc. Linn. Soc. 4: 612-630. *pls.* 48-50. 1900) is the sixth, and includes descriptions of three new species.—E. P. BICKNELL (Bull. Torr. Bot. Club 27: 373-387. 1900), in continuing his "Studies in Sisyrinchium," has revived Salisbury's genus *Hydastylus* to receive the yellow-flowered *S. Californicum*, and has associated with it 11 other species, 9 of which are described as new.—K. M. WIEGAND (*ibid.* 388-391) has described two new species of *Saxifraga* and one of *Primula* from the northwest.—C. V. PIPER (*ibid.* 392-401), in continuing his "New and noteworthy northwestern plants," describes new species of *Amelanchier*, *Potentilla*, *Saxifraga* (3), *Townsendia*, *Erigeron*, *Castilleja* (2), and *Salix* (2).—KATHARINE BRANDEGEE (Zoe 5: 31-35. 1900), in her third paper entitled "Notes on Cactææ," describes two new species of *Mamillaria*, besides giving critical notes on several other forms.—C. WARNSTORF (Hedwigia 39: 100. 1900) has described four new *Sphagna* from Virginia and North Carolina collected by T. H. Kearney.—J. M. C.

MR. HAROLD WAGER has published the results of his study of the fertilization of *Peronospora parasitica*,<sup>6</sup> his conclusions being as follows: the protoplasm of the oogonium differentiates as usual into vacuolate ooplasm and granular periplasm; a receptive papilla is formed on the oogonium in contact with the antheridium and is penetrated by the antheridial tube; the nuclei of the oogonium and antheridium undergo mitosis before fertilization; soon after the delimitation of the oosphere the central body appears, which seems to play some part in bringing the sexual nuclei together; a single nucleus from the periplasm travels towards the central body, coming in close contact with it, and towards it the antheridial tube advances and discharges a single nucleus; fusion occurs while the nuclei are in the resting stage and not until the oospore is nearly ripe; the central body disappears before fusion; the ripe oospore is uninucleate; no difference is observable between the sexual nucleus and those that remain in the periplasm, all probably being potentially sexual; three types of fertilization and oospore-formation in the *Peronosporæ* may be distinguished, as follows: (1) uninucleate oosphere, binuclear fusion, and uninucleate oospore (*P. parasitica*); (2) uninucleate oosphere, binuclear fusion, and multinucleate oospore (*C. candidus*, *C. Portulacæ*, and *P. Ficariæ*); (3) multinucleate oosphere, multinuclear fusion in pairs, and multinucleate oospore (*C. Bliti*).—J. M. C.

A PRELIMINARY paper by Lidforss<sup>7</sup> records some interesting experiments upon the chemotropism of the pollen tube. *Narcissus Tazetta* furnished material for most of the work.

If the pollen be placed in a 5-15 per cent. sugar-gelatin solution and a piece of stigma be added, the pollen tubes soon turn toward the stigma, as is already

<sup>6</sup> Annals of Botany 14: 263-279. *pl.* 16. 1900.

<sup>7</sup> LIDFORSS, B.: Ueber den Chemotropismus der Pollenschläuche. (Vorläufige Mittheilung.) Ber. d. deutsch. bot. Gesell. 17: 236-242. 1898.



known. Lidforss finds that organic acids, formic, acetic, succinic, lactic, tartaric, malic, etc., as well as amides, glucosides, and tannins, do not produce any undoubted effect upon the direction of growth of the tubes. Diastase, however, produces an almost immediate effect, and experiments show that it is the proteid constituent of the diastase which attracts the pollen tube. Further experiments show that carbohydrates and proteids are the substances which influence the direction of growth and indicate that the movement of the pollen tubes is for the purpose of securing nutrition.

The pollen of most Liliaceæ is more sensitive to mineral salts than *Narcissus* pollen, the same diastase preparation which attracts *Narcissus* pollen quickly killing the pollen of *Fritillaria*, but if the salts be dialyzed out the proteid exerts a strong influence upon *Fritillaria* pollen.—CHAS. J. CHAMBERLAIN.

IN AN ADDRESS before the Niederrheinische Gesellschaft für Natur und Heilkunde zu Bonn,<sup>8</sup> Dr. F. Noll<sup>9</sup> makes a suggestion which is likely to prove fruitful, because it not only groups together some previously isolated facts, but seems likely to be the starting point of further investigations. He has been led to believe that the *form* of the plant body itself is a source of both orienting and formative stimuli. As evidence of this he appeals to the fact that in regions of curvature the concave side of roots remains entirely free of lateral rootlets, which are limited to the convex side; or, if they appear at all upon the neutral flanks, they bend more or less sharply toward the convex side. These phenomena have been observed in all the plants studied, including representatives of all the great groups which develop roots.

With these facts Noll correlates Wiesner's "exotrophy," a widespread phenomenon, whereby the growth of external members or their external flanks is conspicuously promoted as compared with that of corresponding members situated toward the interior. Vöchting's "rectipetality," Pfeffer's "autotropism," and the phenomena of replacement, regeneration, and polarity have a new light cast upon them by Noll's suggestion. Many old fields also need exploring from this new point of departure.—C. R. B.

THE DISCOVERY by A. C. Hill that the hydrolysis of maltose by maltase is a reversible process and that this enzyme is also capable of forming maltose from glucose<sup>10</sup> seems to be supported by the results of M. Cremer's investigations.<sup>11</sup> He finds that the glycogen-free expressed extract of yeast is capable of producing glycogen in a 30 per cent. solution of fructose, which is

<sup>8</sup> Sep. Abdr. aus d. Sitzungsber. d. Niederrhein. Gesells. 1900; Sitzung von Jan. 15. 1900. pp. 6.

<sup>9</sup> Noll's extended researches are announced to be published in Thiel's Landwirthschaftliche Jahrbücher early in this year.

<sup>10</sup> Trans. Chemical Soc. 1898: 634-658.

<sup>11</sup> Berichte d. deutsch. chem. Gesells. 1899: 2062-2064.

a still more complex process than the production of maltose from glucose. Meyer suggests<sup>12</sup> that similar relations may exist among other polysaccharides which are hydrolyzed by enzymes. Holding that the enzyme which digests starch has its origin in the plastids,<sup>13</sup> he now thinks it probable that this enzyme acts like maltase. When a concentrated solution of sugar enters the stroma from the cytoplasm, amylose is formed, which on reaching a certain concentration crystallizes out in the form of a starch grain; while if sugar is wanting in the cell a relatively active inversion of amylose occurs, and a rapid solution of the starch grains. The suggestion is interesting and deserves careful testing.—C. R. B.

MR. W. H. LANG has published the second number<sup>14</sup> of his series entitled "Studies in the development and morphology of cycadean sporangia," dealing with the ovule of *Stangeria paradoxa*. His results are as follows: two ovules are developed on each sporophyll; the archesporium consists of a mass of cells, one of which is selected, enlarges very much, and forms a row of three, the lowest cell of which organizes the megaspore; at the time of pollination the sac is full of endosperm (but without archegonia), the pollen chamber is fully developed within the beak-like process of the nucellus, but the breaking down of nucellar tissue between the pollen chamber and the embryo-sac has not begun; as in *Cycas* and *Zamia*, the sperms are large, spirally twisted, and multiciliate, with the blepharoplast band evident; disorganization of endosperm tissue between the pollen-chamber and the embryo-sac gives free access to the latter, and the sperms are discharged into the archegonial chamber of the nucellus, reaching the neck by swimming.—J. M. C.

HUGO DE VRIES has been experimenting in hybridizing the races of maize,<sup>15</sup> producing the well-known phenomenon of xenia. Associating his results with those of Nawaschin and Guignard, who have announced the discovery of double fertilization in the embryo-sac of certain liliaceous plants, he has reached the conclusion that the hybridization of the embryo is always accompanied by the hybridization of the endosperm, and that this phenomenon can only be explained by double fertilization. He found that every grain whose endosperm showed the characters of the pollinating parent had a hybrid embryo; and that every grain whose endosperm showed the characters of the embryo-sac parent had an embryo of pure race, and was therefore self-fertilized. In a few words he puts it that double fertilization is corroborated by double hybridization.—J. M. C.

<sup>12</sup> Bot. Zeit. 57<sup>2</sup>: 313. 1899.

<sup>13</sup> Untersuch. über Starkekörner 169.

<sup>14</sup> Annals of Botany 14: 281-306. pls. 17-18. 1900.

<sup>15</sup> Sur la fécondation hybride de l'endosperme chez le maïs. Rev. gén. de Botanique 12: 129-137. pl. 15. 1900.

GEORGE MASSEE, in discussing the origin of the Basidiomycetes,<sup>16</sup> presents the following summary: (1) in the conidial condition certain ascigerous fungi bear their spores on structures morphologically indistinguishable from the basidia of the Protobasidiomycetes; (2) some members of the same form genera as those described in (1), as *Stilbum vulgare* Tode, have lost the ascigerous condition from their life cycle, and are accepted as true Protobasidiomycetes; hence we are justified in concluding that the Protobasidiomycetes as a group originated from ancestors that represented the conidial condition of ascigerous fungi; (3) there is no evidence in favor of the suggestion that the Autobasidiomycetes are descended from the Protobasidiomycetes; on the other hand, the evidence in favor of the Autobasidiomycetes having been derived by gradual modification of the spore-bearing organs, or basidia of conidial forms of certain ascigerous fungi, is not lacking.—J. M. C.

J. W. TOUMEY has published the results of his study of "crown-gall" as Bulletin 33 from the Arizona Agricultural Experiment Station, under the title "An inquiry into the cause and nature of crown-gall." The name is applied to a disease which appears as fleshy outgrowths on the roots of deciduous fruit trees, usually at the crown. The author concludes that the cause is a specific organism, one of the Myxomycetes, parasitism among which has been thought to be confined to a single species (*Plasmodiophora brassicae*, which causes "club root" in cabbage and allied plants). The plasmodium and its effect upon the host cells are fully described, as well as the sporangia. The study of the organism is made the occasion of the establishment of a new genus, *Dendrophagus*.—J. M. C.

NOW THAT TRIPLE FUSION has been discovered in connection with the formation of the primary endosperm nucleus, illustrations are rapidly multiplying. First announced by Nawachsin for *Lilium Martagon*, it was confirmed for the same species by Guignard and Miss Sargent, the former adding other species of *Lilium* and also *Fritillaria* and *Tulipa*. Now Miss Ethel N. Thomas finds vermiform nuclei wrapped about the polar nucleus in *Caltha palustris*;<sup>17</sup> while Nawaschin has described and figured<sup>18</sup> triple fusion in the embryo-sacs of *Helianthus annuus*, *Rudbeckia speciosa*, and *Phaius Blumei* (an orchid).—J. M. C.

WARNSTORF gives<sup>19</sup> a description of the anatomical characters of a number of the species of *Sphagnum* which have not previously been examined with the thoroughness and minuteness which modern taxonomy demands in this group. The characters are drawn from types, and the examination of

<sup>16</sup> Jour. Linn. Soc. 34: 438-448. pls. 15-16. 1900.

<sup>17</sup> Annals of Botany 14: 318-319. 1900.

<sup>18</sup> Ber. d. Deutsch. bot. Gessell. 18: 224-230. pl. 9. 1900.

<sup>19</sup> Bot. Cent. 82: 7 sq. 1900.

these has led to the reduction of many of the recently proposed species, particularly those of C. Müller, whose later work was so prolific of "new" species. Some of the critical remarks touch American and Antillean species.—C. R. B.

VON DERSCHAU has studied carefully the process of wall thickening in the formation of the teeth of mosses.<sup>20</sup> He finds that preceding the true thickening process, the activity of the cytoplasm consists only of its preliminary accumulation on the membrane to be thickened. In this the nucleus exerts no clearly recognizable control, but does do so in the thickening process itself. This consists of the apposition of materials early produced in the cytoplasm, of which cellulose is the primary one, the other substances being such as promote hygroscopicity and resistance to decay.—C. R. B.

DR. GEORG GOETZ has restudied the development of the egg in the Characeæ,<sup>21</sup> which leads him to consider the group as independent of the algæ and derived from the primitive type of the archegoniates, just as the mosses and ferns. The *Wendungszellen* of *Nitella* he regards as a reduced archegonium wall, and the peculiar separation of a portion of the nuclear substance reminds one of the formation of a ventral canal cell.—C. R. B.

DR. F. NOLL proposes<sup>22</sup> the use of the scape of dandelion for demonstrating the mechanics of tendril coiling. By cutting out from the scape a long strip not much wider than thick, and, after fastening the two ends so that they cannot rotate, immersing the preparation in water, the inner tissues elongate so greatly that the strip is thrown into a spiral coil with one or more points of reversal, thus imitating very closely a hugh tendril.—C. R. B.

ACCORDING to Palladine,<sup>23</sup> though light is not requisite for the regeneration of proteids, if cane sugar is supplied to the etiolated leaves of *Vicia Faba* under experimental conditions, the regeneration goes on more energetically in light than in darkness, and for this the more refrangible light is the more efficient. Such leaves cultivated on cane sugar solution in light respire more than twice as actively as when kept in darkness.—C. R. B.

DR. OSCAR LOEW brings together a useful summary of the present knowledge of the physiological rôle of mineral salts.<sup>24</sup> Unfortunately the greater part of the earlier experimentation in this direction has been more or less misguided, and one must hold very loosely the conclusions reached. This summary, however, will be useful as a guide to the literature of the subject.—C. R. B.

<sup>20</sup> Bot. Cent. 82: 161-168, 194-200. 1900.

<sup>21</sup> Inaug. Diss. Freiburg. 1899. See Bot. Cent. 81: 366. 1900.

<sup>22</sup> Flora 86: 388. 1899.

<sup>23</sup> Revue gén. de Bot. 11: 81-105. 1899.

<sup>24</sup> LOEW, O.: "The physiological rôle of mineral nutrients." Bull. 18, U. S. Dept. Agric., Division of Veg. Physiol. and Path. 8vo, pp. 60. Washington: Gov. Printing Office, 1899.

OF GREAT INTEREST to all botanists is the report of Henry Gannett<sup>25</sup> on the forests of the United States. The author as chief of the Division of Geography and Forestry of the U. S. Geological Survey has had long and intimate acquaintance with the subject. It seems that on July 1, 1899, there were thirty-seven government forest reserves, aggregating 72,139 square miles, composed mainly of mountainous, rugged country, of no value for agriculture, but especially favorable for tree growth. The states containing these reserves are Arizona, California, Colorado, Idaho, Montana, New Mexico, Oregon, South Dakota, Utah, Washington, and Wyoming. Of these Washington has much the largest proportion, 19 per cent. of the total area of the state being thus reserved. The bulk of the publication consists of abstracts of reports on forest reserves by special agents sent to examine them. — J. M. C.

TO THE LIST of seeds whose germination is affected by light, Heinricher adds our common *Veronica peregrina*.<sup>26</sup> Light, even weak light, accelerates germination, as a difference of five to eight days between light and dark cultures strikingly shows. The effect of the light depends apparently upon its action in promoting the digestion of the reserve foods.—C. R. B.

OSWALD RICHTER recommends<sup>27</sup> a concentrated solution of ammonia, used boiling, at 40° C., or cold, as a maceration fluid. He finds it much superior to the usual acids, because the cell wall is always intact. In many cases, also, the cell contents are preserved and even made clearer.—C. R. B.

PROFESSOR DR. S. ROSTOWZEW has devised,<sup>28</sup> apparently independently, the laboratory table with trapezoidal top, identical in form with that considerably used in this country when window space is scant. The form was first suggested, we believe, by Dr. C. E. Bessey.—C. R. B.

<sup>25</sup> Extract from Twentieth Ann. Rep. U. S. Geol. Surv., Part V, Forest Reserves. Pp. 1-37, with 7 maps. 1900.

<sup>26</sup> Ber. d. deutsch. bot. Gesells. 17: 308. 1899.

<sup>27</sup> Oesterr. Bot. Zeitsch. 50: 5-11. 1900.

<sup>28</sup> Bot. Cent. 81: 361. 1900.

## NEWS.

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DR. E. B. COPELAND, assistant professor of botany in the University of West Virginia, has been advanced to a professorship.

DR. HUGO ZUKAL, professor of plant pathology in the Hochschule für Bodencultur in Vienna, died on February 15. (Bot. Cent.)

THE OFFICERS of the Botanical Society of America for 1900-1 are as follows: *President*, B. D. Halsted; *Vice President*, R. A. Harper; *Treasurer*, C. A. Hollick; *Secretary*, G. F. Atkinson; *Councillors*, B. D. Halsted, R. A. Harper, C. A. Hollick, G. F. Atkinson, B. L. Robinson, C. E. Bessey, and F. V. Coville; *Custodian of Library*, W. Trelease.

THE DIVISION of Vegetable Physiology and Pathology has just completed an extensive series of experiments at Halstead, Kan., in connection with work on the development of new forms of cereals by breeding. The work was planned by Mr. M. A. Carleton, but owing to his absence abroad it was carried on by Mr. D. B. Swingle, a graduate of the Agricultural College at Manhattan, Kan.

WM. J. FOX, of the Philadelphia Academy of Sciences, has had the good fortune to discover in the library of the academy a copy of that very rare work of Rafinesque entitled *Western Minerva*, or *American Annals of Knowledge and Literature*. Rafinesque proposed to publish a journal with this title, but only a single number appeared, bearing the date 1821, and, being suppressed by the printer, Rafinesque states that he only saved three copies of it. Mr. Fox gives a very complete account of the contents of the work in *Science* of August 10, 1900. It is a small quarto and contains vi + 82 pages, and is of interest to botanists in that it contains new names for plants which have not yet been noted in synonymy. It is an interesting question whether a work suppressed by the printer, and presumably never distributed, can be counted as a publication. In the copy discovered by Mr. Fox some of the pages are original proof sheets, being printed on one side only, containing corrections, and also such notes as "I must see another proof." It would seem necessary to distinguish between publication and printing, and the *prima facie* evidence is that this discovered pamphlet, although of great interest, does not come within the definition of a publication when the question of priority is concerned. Only four pages are given to botany, and Mr. Fox gives very fully their contents.

## BOTANICAL GAZETTE

OCTOBER, 1900

CELL AND NUCLEAR DIVISION IN FULIGO  
VARIANS.

R. A. HARPER.

(WITH PLATE XIV)

OUR knowledge of such important vital phenomena of the Myxomycetes, as the formation of the spores and the capillitium, and nuclear and cell division, is still based quite largely on the data given in the single paper published by Strasburger (23) in 1884.

Strasburger studied *Trichia fallax*, and his main conclusions were that the nuclei divide karyokinetically just before spore formation, the capillitium is formed in vacuoles, and the spores are formed by simultaneous breaking up of the multinucleated mass along hyaline lines into uninucleated spores. Strasburger found his material on decaying stumps, and speaks of finding all stages in the development of the sporanges simultaneously. Lister mentions also that the sporangia require several days to mature after their first appearance.

Strasburger describes the capillitium, which in *Trichia fallax* consists of long spirally-thickened threads tapering at each end, as arising in vacuoles of the protoplasm. These vacuoles become elongated, and the hollow tubular capillitium thread is formed in their interior. Strasburger describes the wall of the thread as being formed by the fusion of microsomes, which become aggregated in the membrane of the vacuole, and unite to form a thin, transparent pellicle. Further deposition of

microsomes in spiral lines about the so formed thread leads to the formation of the spiral ridge-like thickenings of the mature capillitium. The whole process is identical with that which takes place in the formation of a cell wall, according to Strasburger's earlier accounts of that process.

Whether or not the microsomes are the units in cell wall formation, we have here excellent evidence that the interior protoplasmic surface, which lies next to the vacuole, is equivalent to the exterior surface which forms the peridium of the entire sporangium. Each surface is able in essentially similar fashion to deposit on occasion a resistant membrane over its whole extent. The doctrine of the equivalence of plasma-membrane and vacuolar membrane as developed by Pfeffer and De Vries finds strong support in this method of capillitium formation. Strasburger describes a period of nuclear division as preceding spore formation. The division is karyokinetic and the equatorial plate, separation of the daughter chromosomes, and development of the daughter nuclei are figured. The spindle fibers are inclined only slightly toward each other at the poles. The nuclei all divide at the same time, so that each section shows thousands of karyokinetic figures. Strasburger says little as to the method of spore formation. He figures the cleavage as producing the one-nucleated spores directly, and describes the boundaries of the spores as at first consisting of granules and then of clear lines, and notes also that the differentiation proceeds for the most part from the periphery toward the center. The young spore is at first polygonal, then rounds itself up and becomes enclosed by a wall.

Zopf (27) adheres to the view that the capillitium represents plasma masses (*Hyaloplasma*, *Gerustplasma*) which are not used for spore formation and have become hardened. He does not pretend, however, to have verified this statement for himself. Later (p. 63) he accepts Strasburger's account of the formation of the capillitium in *Trichia*, and is inclined to regard it as true for all the forms with hollow capillitia (*Coelonemata*), while still holding that the forms having a capillitium composed



of solid threads (Stereonemata) form the latter from strands of protoplasm. As a matter of fact, both forms of capillitium may arise in vacuoles, as I have been able to determine in the cases of *Stemonitis* and *Lycogala*. The account given by Strasburger is correct, and I have not thought it necessary to give further figures at this time. Whether the thread is hollow or solid, simple or branched, free or connected with the peridium or a columella, are entirely secondary conditions, depending on the extent and form of the vacuoles.

Massee neglects Strasburger's account of capillitium formation altogether, and advances the extremely loose and erroneous view that generally a surplus portion of the protoplasm takes the form of a more or less complicated network mixed with the spores, and homologous with the strands described as being present in the sporangium of *Mucor*, inasmuch as both structures are made from a substance separated from the protoplasm during spore formation. I have shown (4) that the so-called intersporal protoplasm of the *Zygomycetes* is merely excreted slime, and Strasburger's account of capillitium formation is true for all slime molds that have as yet been carefully investigated.

Lister has discovered karyokinetic division of the nuclei in the sporanges of a considerable number of genera, and has observed karyokinetic figures in the dividing swarm spores and in the growing plasmodia, though he also figures direct division as occurring at this latter stage. He concludes that whenever cell formation occurs in the life history of the Mycetozoa, the nuclei divide by karyokinesis. Lister also observed in certain genera lobed plasma-masses containing six to ten nuclei in the equatorial plate stage, and describes these masses as separating into uninucleated spores during the succeeding stages of nuclear division. For other genera he confirms the account of Strasburger, according to which nuclear division is complete before spore formation begins.

De Bary (1a) follows Strasburger in describing the spore formation for the whole group of slime molds as taking place by simultaneous breaking up of the protoplasm of the sporange

into uninucleated masses, and this is the current statement of botanical text-books.

Rosen (17) made a very thorough study, as he describes it, of *Fuligo septica*. He finds nuclei of two kinds, one poor in content and containing a so-called middle-body, and the other so densely filled with stainable substances as to appear almost homogeneous. The relative number of these two types of nuclei vary at different stages in the development of the slime mold. Nuclear division occurs prior to spore formation, but the process is described as much simpler than in the higher plants. Rosen thinks it belongs to the karyokinetic type, but it is doubtful whether a spindle figure is formed, etc. The cleavage is positively stated to be simultaneous, and to take place by the deposition of a network of granular plates which cut the protoplasm up at once into polyedric uninucleated spores. These plates are said to show microsomes very plainly, the latter being placed at right angles to the plane of the plate.

As will be seen below, my own observations on *Fuligo* have led to entirely different results from those of Rosen. I am convinced that his two forms of nuclei are due to inequalities in fixation such as sometimes occur. As to the method of nuclear division and spore formation I am certain that Rosen failed to find material in the stages when these processes occur. His description of nuclear division must have been based on resting nuclei whose contents happened to be somewhat unusually placed. As for the network of granular plates with microsomes such as he figures, I am convinced that no such structures are to be found in *Fuligo* at any stage of development. The difficulties in the way of obtaining accurate results in the study of fungus cells and nuclei are great, but not sufficient to justify such slipshod results as those of Rosen in the paper under consideration.

A summary of our present knowledge of the Myxomycetes has recently been published by Jahn (9), and reference may be made to it for a further account of the literature of the group.

My material was fixed in Flemming's solution, weaker formula, sectioned, and stained with Flemming's safranin, gentian-violet and orange.

The formation of the aethalium of *Fuligo* has been very well described by De Bary so far as its grosser structure is concerned. The plasmodium which is ready to form spores creeps to the surface of the substratum, and there forms a reticulum which is similar to that of the vegetative condition except that it is more dense. It becomes a rounded cake-like mass, the meshes of the reticulum being relatively small and the protoplasmic strands very thick. We have in fact at this stage a contracted reticulum, the interprotoplasmic spaces having become minute. At this stage the solids which have been imbedded in the protoplasm are all thrown out upon its surface. Large amounts of water containing salts in solution are excreted, and, the water evaporating, the salts are deposited as crystals along with the rejected solids. These waste materials are found in all the meshes of the protoplasmic reticulum, and form a sort of fragile framework piercing the ripe aethalium in all directions. The yellow coloring matter of the plasmodium is also transferred to these waste materials, so that the protoplasm is left apparently homogeneous and colorless.

A further step in forming the aethalium consists in the continued contraction of the protoplasmic reticulum, so that its superficial strands are withdrawn toward the center. In this withdrawal of the protoplasm from the peripheral parts of the mass the excreted wastes are left behind, and form thus a porous friable crust over the surface of the protoplasmic mass. At the margins of the aethalium this waste material frequently takes the form of a thin membranous almost papery border. In this further contraction of the protoplasmic reticulum the interprotoplasmic spaces become reduced in many cases to mere strands or plates of the yellow colored waste materials described above. In other cases the spaces remain as oval or angular lacunae lined with a thin yellow crust of the same excreta. *Fig. 1* shows a section through a portion of an aethalium relatively free from

such cavities. In *fig. 2*, from another portion of the same aethalium, parts of several lacunae are shown, and their relative size and distribution is thus partially indicated.

In addition to the solid particles and solutions thrown out, the protoplasm excretes over its whole surface a thin fragile membrane, in which the crystals of lime are frequently partly imbedded. This membrane is by no means as thick as in the case of the slime molds which produce sporanges, but it appears very clearly in microtome sections. In most regions it is hardly more than a cement to hold together the lime crystals in a continuous film. In other regions, where these are less abundant, it appears as a very thin homogeneous membrane. It lines all the interprotoplasmic cavities mentioned above, as well as covering the peripheral portions of the protoplasm. It is always next to the protoplasm itself. I have never found crystals or other solid excreta between it and the plasma membrane.

At a stage when cleavage is just beginning, such as that shown in *fig. 1*, the nuclei are generally in the resting condition, and are distributed rather unevenly through the cytoplasmic mass. Frequently they appear aggregated in rather dense groups in certain regions, while in adjacent regions of the cytoplasm they are less numerous. Spore development now begins with the formation of cleavage furrows, which usually arise first on the external surface of the entire aethalium and cut down at all angles into the homogeneous protoplasm. These furrows are very narrow and sharp in some cases, and quite widely opened in others (*fig. 1*). This latter condition may be due, at least partly, to a slight shrinkage in fixation. Very commonly they are curved and forked so as to cut off a superficial layer of segments. Almost simultaneously with the formation of these furrows on the surface of the entire aethalium, similar furrows are formed on the surfaces of the lacunae of the contracted protoplasmic reticulum as described above. These surfaces, of course, are in reality external surfaces of the protoplasm, and the formation of cleavage furrows from them is not in any sense to

be compared to the cleavage from vacuolar surfaces as I have described it in *Pilobolus* (4).

As noted above, the first cleavage furrows commonly do not cut through the entire mass of protoplasm in which they are formed, but curve and fork so as to cut off one or more superficial layers of segments. Further furrows, not continuous with the first, then cut through the central mass, dividing it up into large blocks, each with many nuclei. Meanwhile the superficial segments have still further divided, so that we regularly have one or few nucleated masses at the surface, while the central protoplasm is relatively undivided. The segmentation is very plainly a progressive process proceeding from the periphery toward the center. There is no such thing as a simultaneous breaking up of the protoplasm into uninucleated fragments. The protoplasm which thus segments is quite homogeneous, as noted above. There is no differentiation of hyaline zones or other specialized regions prior to the formation of the cleavage furrows. Furthermore, the nuclei show no special distribution about the cleavage planes. As seen in *figs. 1* and *2*, it is quite common to find a group of nuclei on one side of a cleavage furrow while they are lacking over a considerable area on the opposite side. There is no indication whatever at this stage that the nuclei exert any direct influence on the orientation of the cleavage planes.

If we examine the protoplasm immediately in front of one of these cleavage furrows also, we find it without differentiation of any sort which would indicate the direction which the furrow will take. Aside from the fact that it is common for the cleavage to advance along the same plane or curve in which it has started, it is quite impossible to predict in the case of any unfinished furrow, *i. e.*, one which has not yet cut through the protoplasm in which it lies, what direction it will take. It is very noticeable that these cleavage furrows do not necessarily cut the mass which is segmenting through its shortest axis, any more than through its middle. It is very common to see a strip or sheet cut off from the side of a larger mass in such a fashion that the plane of cleavage lies in the long axis of the mass which is

divided. I have even found some evidence that sausage-shaped masses are cut out of the center of larger masses by means of a cylindrical cleavage furrow. It is very common to find semi-cylindrical masses cut from the surface of the protoplasm by two furrows which curve toward each other so as to form a trough-shaped cleavage surface. All of the above varieties as to form and direction of the cleavage furrows are illustrated in *figs. 1-4*.

At and immediately prior to the time when cleavage commences in Fuligo, its nuclei are all in the resting condition. None of the nuclei indicated in *fig. 1* were dividing. A very little later, almost simultaneously with the formation of the first superficial cleavage segments, the nuclei throughout the entire aethalium begin to divide karyokinetically. In some cases it may be that the peripheral nuclei commence to divide earlier than those which lie deeper. But the difference, if it exists, generally is a very slight one. On the other hand, the process of division seems to begin progressively rather than simultaneously in different parts of the aethalium, regardless of depth from the surface. This is shown by the fact that in examining sections different stages of karyokinesis are found in different parts of the same section. For example, all the nuclei in a certain region a few hundredths of a millimeter in diameter may be in the equatorial plate stage. Moving from this region in one direction one will find a gradual transition to the anaphase stages. Moving in another direction one may find prophases, or one may find nuclei in anaphase on all sides of a region showing only equatorial plates. There is no constancy in the order of stages which will be found in moving from the peripheral to the central or deeper portions of a section cut radially to the surface of the oval, cake-shaped aethalium. It is an absolute rule, however, that widely separated stages in division are never found in close proximity to each other, at least in continuous masses of protoplasm; and generally, passing over one of the numerous lacunae, which, as noted above, pierce the aethalium in all directions, does not involve any sudden transition in the

stage of nuclear division. We may assume that the nuclei begin to divide at numerous isolated points in the aethalium, and that the nuclei of adjacent regions begin their division progressively in all or only in certain directions. If we consider that the division begins in response to a stimulus either external or internal, we should imagine the stimulus being propagated in one or several directions, from the point of its first effectiveness. Strasburger has remarked upon this same wave-like progress of the tendency to nuclear division in *Trichia*, and has compared the phenomena there with those in the division of the nuclei in the young endosperm of *Fritillaria*, in which the nuclei at one end of the embryo sac begin to divide first, and the process is then taken up progressively by the successive nuclei through the whole length of the endosperm layer.

Nuclear division proceeds thus during the whole process of cleavage, but without any relation whatever to the latter process. Karyokinetic figures can be found oriented in all possible ways to the cleavage furrows described above, and any stage in division can be found in segments of the dividing cytoplasm of any shape or size, as will be seen from *figs. 4-9*. As a further example, segments can be found with a single nucleus in any stage of division (*figs. 11, 13, 15, 16*). The details of these nuclear divisions, so far as I have been able to work them out, will be described below.

The time relations of nuclear and cell division in *Fuligo* are thus seen to be entirely different from those in *Trichia* as described by Strasburger. In *Trichia* nuclear division is complete before cell division begins, while in *Fuligo* the two processes are carried on simultaneously. The difference may be associated with a necessity for more rapid ripening of the fruit body of *Fuligo*. According to my observations, the building of the aethalium and formation of the spores takes place within twenty-four hours in the case of *Fuligo*. Strasburger does not state just how long a time is required for the development of the sporangium of *Trichia*, but Lister found that they require from two to four days to ripen after their first appearance.

We may return now to the further consideration of the cleavage processes by which the aethalium is cut up into spores. We have noted above that the primary cleavage furrows cut into the surface of the protoplasmic mass at varying angles, that they may be curved, may branch, etc., in the most irregular fashion, with no reference whatever to the distribution of the nuclei. This much of regularity, however, can be seen. The furrows are so oriented with reference to each other and to the surface of the mass that cleavage at first progresses more rapidly at the surface than in the center. Thus, one or more layers of very irregular one- to several-nucleated segments are cut off on the periphery, while the central mass has been cut through by only a few furrows. What is true of the exterior of the aethalium as a whole is also true of the surfaces of the interprotoplasmic gaps or lacunae. It can be seen from *fig. 2* that the surface of each such lacuna is lined by a layer of one- or few-nucleated segments, while beneath them larger multinucleated segments are found. The peripheral segments are very irregular in shape, as are also the larger central segments. Frequently broad thin plates are found; elongated sausage-shaped masses are also common. As noted above, the cleavage planes follow no such simple rules as cutting through the short axis of the mass to be divided, or always dividing a mass successively in planes that intersect at right angles. Hofmeister's law, also, that cell division always occurs transversely to the axis of most vigorous growth, has no application here, since no growth is taking place at the time when these divisions occur.

If we study the cleavage of any one of these central masses of protoplasm we shall find the orientation of the furrows essentially similar to that of those which cut in from the surface of the entire mass. *Fig. 5* shows such a mass with its nuclei all in the equatorial plate stage, both polar and profile views of the latter being shown. It is to be especially noted also that the furrows in *fig. 5* are not directly continuous with those that appeared first on the surface of the mass from which the segment in question was taken. No single furrow can be traced



for any great distance in an unbroken plane or curve. The furrows which are to further subdivide the segment represented in *fig. 5* have been formed independently of those by which the block itself was delimited. The plan seems to be that each furrow may cut through the mass in which it originates, but may not continue across the furrows with which it intersects so as to cut through successive masses in any specific direction. There are no general planes of cleavage for the whole mass. As noted already, the first furrows that form do not as a rule cut deep down into the mass toward a center. Rather they branch or are curved so as to cut off irregular blocks on the surface. New furrows forming on the surface of these, and at very varying angles with them, continue the cleavage into the deeper portions of the mass.

It is well shown in *fig. 5* that the furrows at this stage also cut into a perfectly undifferentiated mass of protoplasm, there being absolutely nothing by which to predict the path they will take except the general direction which they have already entered upon. The protoplasm is singularly homogeneous, without large vacuoles or inclusions of any sort, and through this undifferentiated mass these furrows are formed. It is further seen that they may be either plane or curved, and lie at various angles with each other and the surface of the mass.

This type of cleavage results in no very definite conditions as to the size of the segments formed. Still we can find a stage when the peripheral segments of the mass are quite regularly uninucleated and the deeper portions have been cut to various dimensions, each containing from eight to sixteen nuclei. So far the process resembles that already described for *Synchitrium*; but at this stage a very noticeable difference in the method of division makes its appearance. This difference is shown in *figs. 6-9*. Whereas, hitherto, there has been absolutely no differentiation of the protoplasm to mark the path to be taken by the cleavage furrows, now broad hyaline areas are formed midway between each pair of dividing nuclei. These are not at all hyaline zones of equal thickness, but furrows broader at the surface

and narrowing toward the centers between each pair of nuclei. The surface is generally slightly depressed in the line of these hyaline regions, indicating the beginning of the furrow which is actually to sever the portions thus preliminarily marked off by the hyaline regions.

The appearance is as if all the denser portions of the protoplasmic mass had contracted about each nucleus as a center, thus leaving irregular, furrow-shaped, less dense spaces in the middle region between each pair of nuclei. These areas contain very little or no stainable material, and seem to be filled with a watery liquid merely. They are, however, not in any sense rounded vacuoles whose cell sap shows surface tension where it comes in contact with the denser protoplasm. The surface of the rounded mass of protoplasm aggregated about each nucleus is by no means smooth and even, as is the surface of the protoplasm about a vacuole. The denser protoplasm passes over by insensible gradations into the less dense material in a fashion very hard to reproduce in a drawing. Peripherally these hyaline areas are bounded by a very thin protoplasmic film consisting of little more than the plasma membrane itself, which can here be more perfectly recognized as a distinct membranous film than in any other condition of cell development which I have yet observed. The plasma membrane is in these stages never broken through. It always forms a perfectly continuous enveloping layer surrounding the entire segment which is being divided, as is shown in *figs. 6-9*. In addition to the existence of these hyaline areas which predetermine the future cleavage planes, another striking condition is to be noticed. The hyaline regions in *Fuligo* bound off in every case a single nucleus and never a group of nuclei. This nucleus may be in process of dividing, but the daughter nuclei are never completely reconstructed at a stage when these hyaline regions are present cutting off the pair. The segregation is about the nuclei as units, and the cleavage thus predetermined is to be a cleavage by which the entire mass will be cut into uninucleated segments. Whereas hitherto the cleavage planes in these central masses have been

oriented without especial reference to the distribution of the nuclei, thus cutting off larger and smaller segments with more or fewer nuclei, they are now to proceed midway between each pair of nuclei, so that equal and uninucleated masses will result. As already noted, the impression is very strongly given by these dense rounded masses separated by relatively watery regions that a contraction has taken place about each nucleus as a center. Such conditions might be produced by a contraction originating in the structure of the cytoplasm itself, or in a pull exerted upon the cytoplasm by the nucleus. After the formation of these hyaline areas, the cleavage is completed by the furrowing of the plasma membrane along the lines marked out. A later stage than that shown in *fig. 6*, in which certain furrows have already cut deeply down into the hyaline regions, is shown in *fig. 7*. The furrows are apparently formed just as they were in the earlier stages, but they follow the hyaline areas, and thus the separation of the uninucleated masses is completed. Such cleavage as this is progressive in the sense that both the hyaline regions and the constriction furrows are developed gradually from the surface inward. When complete, however, it results in the simultaneous production of a number of uninucleated cells equal to the number of nuclei in the original mass. And in this respect it differs markedly from the cleavage in the earlier stages, in which larger multinucleated masses are progressively cut up into segments with fewer and fewer nuclei. I have already figured a similar differentiation of a hyaline region predetermining the plane of cleavage in the formation of the spores of *Pilobolus* (4, *pl. 25, fig. 21*). In this figure resting nuclei are shown in two groups, between which a less dense zone is formed, through which later, as is shown in *fig. 22*, a cleavage furrow passes. The appearance was little regarded in my description of cleavage in *Pilobolus*, but its appearance at a similar late stage in the cleavage of *Fuligo* indicates that it may have considerable significance in connection with the relations of the nuclei to the cleavage phenomena. The hyaline areas, in *Fuligo* at least, appear at about the stage in cleavage when the furrows

seem to be more definitely oriented with reference to the distribution of the nuclei. At the stage of embryonic growth, when they are found in *Pilobolus* also, cell division and nuclear division are proceeding in a somewhat definitely correlated fashion. It can hardly be questioned that whereas in earlier stages the cleavage was largely independent of the nuclei, it comes later to be directed solely with reference to their distribution, and it seems not unnatural to assume that in this latter stage the nuclei control the orientation of the cleavage planes. If this is the case, it is quite possible that the formation of the hyaline zones is the visible expression of this activity of the nuclei.

On the other hand, it is quite possible to assume that cleavage throughout is controlled by the cytoplasm, at first with little reference to the distribution of the nuclei, but later with special reference to the formation of uninucleated cells. The formation of hyaline zones preceding the cleavage furrows might in this case also mark the transition from the earlier irregular to the later more definitive stage of cleavage without implying any special activity of the nuclei. I have already noted that it is quite as easy to assume that the cytoplasm itself contracts about the nuclei as that it is drawn together by a tension exerted from the nuclei. Either view is consistent with the assumption that material for the growth of the plasma membranes is formed in the nucleus and passes outward from it to the newly-forming cell boundaries. As is seen from *figs. 6-9*, the nuclei are dividing while the cleavage just described is going on, so that the uninucleated segments formed become almost immediately binucleated. Cell division then follows either before or after the complete reconstruction of the daughter nuclei (*figs. 16, 17*). Thus, in the end, uninucleated spores are produced. The formation of a hyaline region and constriction furrow for the division of a binucleated cell whose nuclei are already in the anaphase stage is shown in *fig. 9*. The beginning of the constriction for the final division of a binucleated cell to form two uninucleated spores, the hyaline region having not yet appeared, is shown in *fig. 16*.

Transition types of cleavage between that without and that with a preliminary formation of a hyaline furrow are abundant. *Fig. 8* shows a six-nucleated mass of protoplasm dividing by furrows, which in three cases are preceded by hyaline differentiation, and in the other two cases are cutting directly into the undifferentiated protoplasm.

With the formation of uninucleated segments whose nuclei divide no more, the process of cleavage is complete. As noted above, the cleavage results in uninucleated segments at the periphery of the protoplasmic masses much earlier than in their interior. The nuclei in these early formed segments are always found dividing. The definite spore cells with a single resting nucleus are probably formed first in those regions of the aethalium where cleavage first began. The final delimitation of the spores seems to proceed progressively from these regions in all directions through the aethalium. Fully formed spores may ultimately be found throughout the greater part of the aethalium, while in certain regions here and there cleavage may still be in progress. There is, however, in the later stages of cleavage no such marked difference between peripheral and central regions as there was during the early stages. Whether this is due to retardation in the cleavage at the periphery during the later stages, or whether the nuclei there divide repeatedly to prolong the process, I have not been able to determine.

Summarizing, we may characterize the whole process as one of progressive cleavage by means of furrows which cut through the protoplasmic mass in very many directions and at very varying angles to each other. The process is progressive both in that the furrows originate on the surface and proceed gradually toward the center, and in that larger multinucleated segments are first formed which are by further divisions reduced to the condition of uninucleated spores. It may perhaps be distinguished from bipartition as a process of successive multipartition, since cleavage furrows may invade any particular portion of protoplasm simultaneously from a number of directions. At first the orientation of the cleavage planes shows no evident relation

to the distribution of the nuclei. Later the furrows proceed in every case so as to cut off uninucleated masses approximately equal in size. This later period of cleavage is characterized in many cases by the aggregation and rounding up of the denser cytoplasm about the nuclei so as to leave hyaline regions midway between each pair of nuclei, thus predetermining in each case the plane of cleavage to be followed later by the cleavage furrow. This type of cleavage results immediately in every case in the formation of uninucleated cells whose nuclei, however, may still be in a state of division. In the end, the entire protoplasm of the aethalium has been cut into uninucleated segments which are at first naked bits of protoplasm. Later each cell becomes surrounded by a wall and constitutes a spore.

Turning now to the phenomena of nuclear division, we may note first of all that the structure of the resting nucleus conforms, in spite of its small size, to that of the nuclei of other fungi and the higher plants. Nuclear membrane, chromatin (nuclein), and nucleole are present, and are differentiated by staining with safranin, gentian-violet and orange, just as sharply as they are in the pollen mother cells of the lily. The nucleole frequently lies in a clear space (*fig. 10*), as is so frequently seen in the nuclei of the root-tip of the onion.

The nuclei, however, are too minute for the successful study of the prophase in spindle formation. In the equatorial plate stage (*figs. 3, 5, 11*) the spindle is sharply differentiated. It shows rather sharp-pointed poles which may be more densely stained at their tips. Broad-poled spindles, such as those figured by Strasburger for *Trichia*, are not found in *Fuligo*. The chromosomes stain deeply in the equatorial plate stage. In polar views it is possible to count the number with considerable definiteness. The great number of these figures to be found in sections of *Fuligo* at this stage make the material especially favorable for such study. The chromosomes are relatively short and thick, and form a very regular equatorial plate, all of them lying practically in a single plane, so that in polar views they are practically all in focus at once. From a study of a large number of

such figures, I am quite certain that the number of chromosomes is twelve.

All stages of the separation of the daughter chromosomes and their migration to the poles of the spindle can be found in the greatest abundance (*figs. 6, 8, 12-15*). The spindle becomes slightly elongated during this process. Connecting fibers are present and form a figure closely resembling that in the corresponding stages in the lily or larch. As the chromosomes first separate, relatively large gaps are seen between the connecting fibers, which appear bunched together in a few large strands (*fig. 13*). The whole connecting spindle is markedly barrel-shaped at this stage. As the chromosomes approach the poles, the connecting fibers become more evenly distributed, and are straightened so as to form a cylindrical series extending between the groups of daughter chromosomes (*fig. 14*). A marked difference between the nuclear divisions in *Fuligo* and those in the asci I have studied is seen in the arrangement of the daughter chromosomes as they are drawn back to the poles. In the asci these chromosomes are widely scattered on the spindle at this stage, some having nearly reached the poles, while others are much nearer the equatorial region. This condition makes this stage the most favorable for counting the chromosomes of the nuclei in the ascus. In *Fuligo*, on the other hand, all the daughter chromosomes retreat simultaneously toward the poles, as is seen in *figs. 6, 13, 14*. They also become quite densely massed together, so that the individual chromosomes are not so easily distinguished in polar views at this stage as in the equatorial plate stage.

As the daughter chromosomes reach the poles, the whole figure is still more elongated, the connecting fibers being drawn out (*fig. 15*) into a long slender strand, which gradually disappears. The poles of the spindle can be distinguished beyond the groups of daughter chromosomes till a very late stage (*figs. 9, 15*).

The nucleole of the parent nucleus in *Fuligo* disappears at a rather early stage as compared with other fungus nuclei. It is

never to be found lying midway between the daughter nuclei near the old spindle, as is regularly the case in many asci. The daughter nuclei are apparently reconstructed in the ordinary fashion. The figures, however, are too small to show very characteristic details at this stage.

The similarity of the whole process of nuclear division in its main outlines here to what is found in higher organisms is certainly very striking, and shows clearly enough that simplicity of structure and life history on the part of the whole organism is by no means to be taken as indicating a corresponding reduction in the complexity of the nuclear structures and activities.

The capacity of the slime molds to become encysted at any stage in their life history when conditions become unfavorable is very well known. A condition which I have sometimes found, and which is represented in *fig. 19*, indicates that this may occur midway in the process of cleavage. The aethalium in question was made up of rounded, two- to several-nucleated masses, each provided with rather a thick wall. Whether later with a return of favorable conditions such masses would continue their cleavage, and form normal uninucleated spores, or whether they would themselves function as spores, I have not been able to determine. A normal uninucleated spore is shown in *fig. 18*.

The aethalium and the sporanges of the Myxomycetes differ from the sporanges of Synchronium, Pilobolus, and Sporodinia, whose method of spore formation I have already described (4), in that the multinucleated condition in the former originates at least in the formation of the plasmodium. The plasmodium is a product of cell fusions without nuclear fusions, so far as known at present. Physiologically considered, in all its functions of nutrition, growth, and response to external stimuli, it is the equivalent of such multinucleated masses of protoplasm as are formed simply by growth and nuclear division without cell division. The plasmodium itself increases its original volume, as formed by fusion, by this same type of growth. Fundamentally considered, it is the physiological equivalent of the multinucleated mass formed in Synchronium by the division of the original



single nucleus of the vegetative body. The internal relations of nuclei and cytoplasm cannot be conceived as different, merely because in one case the nuclei and separate cytoplasmic masses were brought together by fusion, while in the other they were formed from a uninucleated cell by growth and nuclear division.

Morphologically, however, the two structures must be regarded as entirely distinct, and the possession of the plasmodium, and the capillitium formed as a deposit in vacuoles by the slime molds, is probably sufficient reason for regarding them as constituting a separate developmental series running back to an origin independent of any of the existing groups of algæ or fungi. Sachs (18) has quite recently expressed the opinion that they are to be classed with the fungi, but he brings no morphological evidence to support his view. There can be no question that the Acrasieæ represent simpler forms out of which the Myxomycetes have developed, and we thus have a developmental series leading from simpler to more complex forms. The plasmodium and capillitium, appearing only in the more specialized members of the group, are plainly secondarily acquired structures developed as additions to the structural features of the Acrasieæ, and are not to be directly homologized with physiologically equivalent structures in other groups.

The physiological equivalence of the plasmodium and the multinucleated masses of protoplasm found in other fungi, such as *Synchytrium*, *Pilobolus*, etc., can hardly be questioned. Elsewhere (3) I have discussed the question as to whether these multinucleated masses should be classed as single cells, or as the equivalents of many-celled tissues or organisms. The plasmodium of a slime mold is well calculated to furnish further evidence on this point. In its method of origin by the fusion of distinct amoeboid swarm-spores, it would seem to testify to its multiple nature; still, as noted above, the whole physiology of the plasmodium in its nutrition, reactions to stimuli, and growth, shows most conclusively that it is a unit in exactly the same sense as is an amoeba, or one of the swarm-spores which combined to form it. In fusing, the swarm-spores gave up their individuality

to become parts of a larger mass. That this fusion did not involve a fusion of their nuclei cannot be considered as altering the result so far as the question of individuality is concerned. Where sexual cells fuse and their nuclei unite there is no question that the resulting fertilized egg is a single cell. If, as Häcker (2) has shown is the case in Cyclops, the pronuclei remain distinct through the early cleavage stages of the egg, this cannot be taken as evidence that the two nucleated bodies thus produced are not single cells rather than the equivalents of tissues. These binucleated cells, functionally and morphologically considered, are the equivalents of the later cells of the Cyclops which appear with a single nucleus. The conclusion must be, as I have already pointed out, that the individuality of the cell is independent of the number of nuclei which it contains.

Hertwig argues for the *potential* equivalence of multinucleated cells and tissues. The word potential here of course may mean much or little. In support of his view he urges the case of the insect egg, whose nucleus divides to form hundreds of daughter nuclei before cleavage begins. Later the multinucleated yolk mass is by cell division separated into a blastoderm of as many cells as there were nuclei present. It is quite plain, says Hertwig, that the apparently simple egg could not with a single stroke, as it were, have become a multicellular organism. The question here, of course, is how great a change is involved in the transition from the one-celled to the many-celled condition, and on this point it is interesting to note that up to the stage when cell division takes place in the insect egg there has been no visible differentiation of embryonic structures in the egg. The cell division simply transforms the one cell into a mass of equivalent cells, and this need hardly be considered as a change too great to be due entirely to the cleavage process. The relation of multinucleated and uninucleated cells is well shown in the very fact that the visible differentiation of the insect embryo, aside perhaps from the determination of its axes, which was accomplished even earlier, begins after the division of the egg into numerous cells, and not while it remains a single cell, although

it has meanwhile become multinucleated. It would seem that differentiation was dependent to a certain degree, at least, on the interaction of individualized protoplasmic units, each capable of receiving and reacting to independent stimuli as the parts of a multinucleated cell cannot. Hertwig, in his doctrine of biogenesis, himself insists on the importance of the interaction of separate cells for the production of physiological differentiation and division of labor.

To be sure, we have abundant evidence that the multinucleated cell can achieve a certain degree of differentiation, as is shown in the numerous Siphonæ which mimic in their root-like, leaf-like, and stem-like structures, the analogous parts of the higher plants. It is perfectly apparent, however, that this differentiation is on a far simpler scale than is seen in the complex mechanical and other tissue systems and organs of the higher plants. Indeed, the relative unimportance of the Siphonæ as a part of the earth's vegetation is to be regarded as very strong evidence that the type of structure which they show in their multinucleated cells is by no means well adapted to develop complexity and differentiation of structure such as is necessary to meet the manifold variations in environmental conditions to which all plants are subjected. These Siphonæ are after all hardly more differentiated than the infusorians, which are typically unicellular. Pfeffer (16) puts the case very strongly when he points out that we can conceive of no such independent units in the multinucleated cell as the energids of Sachs are defined to be. If the energid is a nucleus with a portion of cytoplasm under its immediate control, there can be no such structures in the Siphonæ, since the protoplasm of their cells is constantly streaming from one point to another, with the exception of the plasma membrane, which remains fixed. No nucleus could thus have any definite relations with any particular portion of the plasma membrane, and it is hard to conceive that in this streaming motion any portion of the semifluid cytoplasm should remain constantly in connection with any particular nucleus. As Pfeffer says, we must conclude that any specific mass of cytoplasm in a

multinucleated cell will be simultaneously influenced by various nuclei which are in contact with it. There can be no invisibly bounded units in which the same living substance remains united. Pfeffer also justly objects to Sachs's characterization of the Siphonææ as *noncellular* plants, and regards them as both morphological and physiological units.

If we compare the method of spore formation in *Fuligo* with that which I have described elsewhere (4) for *Synchytrium*, *Pilobolus*, and *Sporodinia*, it will be seen that the processes in all these forms are identical in their main features, while differing in a number of important details. In the four cases the cleavage is progressive from the surface inward, larger segments being first formed, which are later cut up into uninucleated cells, except in *Synchytrium taraxaci* and *Sporodinia*, in which the multinucleated segments function directly as spores.

In the earlier stages of cleavage in *Pilobolus* and *Fuligo* the furrows pierce through perfectly undifferentiated and quite homogeneous protoplasm, while in the later stages the differentiation of hyaline areas, wedge-shaped in transverse section and cutting through the masses to be divided, predetermine the planes of the cleavage furrows. Such hyaline areas were not observed in *Synchytrium* or *Sporodinia*. In *Synchytrium* and *Sporodinia* nuclear divisions precede cleavage. In *Pilobolus* nuclear divisions occur during the later stages of cleavage, and in *Fuligo* nuclear divisions and cleavage proceed simultaneously throughout.

*Fuligo* is the only one of the five forms in which the uninucleated segments formed by the completion of the cleavage process, and which I have called protospores, become the functional spores directly without further growth or nuclear division. In this respect perhaps the cleavage of *Fuligo* represents a more simple primitive type than that of either of the others.

In all forms the orientation of the furrows with reference to the surface of the dividing mass and with reference to each other is extremely varied, and it can be laid down as a general rule for the forms studied that no one furrow can be traced

continuously through the entire sporangium or aethalium which is to be divided except, perhaps, in the case of the very thin layer of spore plasma in Sporodinia. On the contrary, by the curving and branching of the furrows, segments irregular in their size, shape, and number of nuclei are cut off successively from the periphery toward the center. These segments in turn, and also progressively from periphery to center, are cut up by new furrows into smaller segments, until finally in *Synchytrium decipiens*, *Pilobolus*, and *Fuligo* the uninucleated condition is reached. No general system of cleavage planes, either parallel or radial to the surface of the dividing mass, can be discovered. The path of the cleavage planes as division progresses becomes an inextricable confusion of zigzag lines, branching and intersecting at almost every angle. The occurrence of such similar types of cleavage of the multinucleated mass as are found in the aethalium of *Fuligo* and the sporangia of the *Phycomycetes* must be regarded as another example of parallel development in structures not phylogenetically connected. The explanation of the similarity in these forms of cleavage is to be sought in the fundamental physiological properties of protoplasm, and not in hereditary transmission to the different branches of a series of genetically related forms.

With the above account of fusion in *Fuligo* representing the *Myxomycetes*, types of all the main groups of fungi producing asexual spores in the interior of mother cells have been described except the *Oomycetes*, and while it will be necessary to investigate representatives of all the genera, at least, in these groups, still the hypothesis is fairly justified that some form of progressive rather than simultaneous cleavage by cell plates will be found in every case. Klebs's (15) investigations of *Hydrodictyon* also indicate that the formation of zoospores is at least not by simultaneous division into uninucleated segments, and the whole process in this alga should be further investigated, especially with reference to the occurrence of the nuclear fusions which Klebs describes as occurring in the developing zoospores. It may be noted here also that Bachmann (1), in describing a new species of *Mortierella*, has observed incidentally the marking off of the

surface of the sporangium into irregular polygonal areas at the time when spore formation is beginning. The lines marking off these areas are doubtless the beginnings of the cleavage furrows as I have described them for *Pilobolus* and *Sporodinia*. Bachmann made no sections, but concludes that the spore formation must be a progressive process.

A sufficient number of forms has been investigated to show that progressive cleavage is a widely spread phenomenon among the lower plants, occurring in very many cases when multinucleated masses are to be cut up into smaller cells. That simultaneous cleavage may also occur is quite possible, but the evidence for it is not strong except, perhaps, in the case of the sporangium of the *Saprolegniaceæ*.

I have already shown elsewhere (4) that the progressive cleavage of the sporangium in spore formation is in principle the same process as that which has been described as division by constriction in *Cladophora* and the conidiophores of the mildews, and it will be of interest to attempt a comparison of this progressive cleavage with cell division as found in the growing points of the higher plants, especially from the standpoint of the more general theories of cell division.

Schleiden's (21) doctrine that the form of a plant is determined by its cellular composition, including as the two important factors the arrangement of the new-formed cells in growing regions and the subsequent varying growth and enlargement of these cells in their three dimensions, was first opposed by Hofmeister. Hofmeister (7 and 8, p. 129) advanced the view that cell-formation is subordinate to the growth of an enlarging organ taken as a whole. He held that the growth of the single cells of a vegetative point is controlled and conditioned by some formative principle which determines the growth of the entire organ, this latter being directed toward simple enlargement or the development of some predetermined form. According to this view, growth of the vegetative point cannot be interpreted as determined by the sum of innate growth tendencies of the individual cells.

Hofmeister believed that cell division takes place according to a simple mechanical law. The new cell wall always cuts the axis of most intense growth at right angles (8, p. 127). It is plain that such a principle as this can have no application in interpreting the division of the masses of protoplasm in sporanges whose growth is complete, the accompanying tensions, as may be fairly assumed, having also reached a condition of equilibrium.

Sachs (20 and 18, p. 22) follows Hofmeister in regarding the growth and division of the single cells as subordinate to the growth of the vegetative point as a whole. From a study of the arrangement of the cells in the growing point of the higher cryptogams and flowering plants, he has developed the law of the rectangular intersection of cleavage planes in the successive cell divisions. He regards this as the most universal law of structure in the plant world, and holds that it is independent of all phylogenetic relations, and not a result of natural selection. As is well known, he holds that the outer form of the growing organ is the primary determining factor for the orientation of the cleavage planes. The periclinal planes conform directly to the surface of the embryonic organ. The anticlinal planes cut the periclinal planes at right angles, and if division is to occur in three dimensions the transversals also appear in a third plane at right angles to the other two. Sachs believes that the relations thus expressed are of so fundamental a nature as to be comparable to those determining the relations of the axes of a crystal. The cellular structure of a young organ is related to these *Leitlinien* as the structure of a crystal to the arrangement of its faces and their intersecting angles. The form of a growing point is determined by phylogeny, and selection when given the direction of the cleavage planes, is at once known. Such fundamental relations as these, occurring in the most widely separated groups, are to be considered as innate in protoplasm. Sachs proposes to call them mechanomorphoses. The principle thus developed by Sachs has been generally accepted as explaining the arrangement of the cell walls in the growing points of the higher plants,

and, as Sachs himself notes, the investigation of the apical cell and its divisions has ceased to be regarded as affording a key to the explanation of the development of shoots.

Assuming the correctness of Sachs's law for the higher plants, if we attempt now to apply it to the case of the cell-division in sporanges we are confronted with many difficulties.

First of all, these sporanges do not divide by successive bi-partitions as do the cells in the growing points referred to. Nor do they divide by simultaneous delimitation of the energids which, according to Sachs's view, compose them. Their cleavage is progressive, and the cleavage planes, as shown in sections, form no great series comparable to the anticlines and periclinal planes which Sachs finds in a section of a root tip. In the cleavage of the spore the principle of rectangular intersection is violated constantly, the angles of intersection of the cleavage planes showing no constancy whatever. It may be objected that the spore is not a growing organ, and hence its method of division should not be expected to conform to that of growing points. Growth is complete in the spore before division begins, though it may recommence in the later stages of cleavage in *Pilobolus*. The process in the spore consists in cutting up into cells a mass of protoplasm whose form has been already determined and its growth completed. Still, although Sachs states the principle of rectangular intersection for growing points, and conceives it as determining the arrangement of the cells in the growing point as it pushes forward in the elongation of the shoot, he always conceives the divisions as occurring in these growing points after the essential embryonic growth of the cell concerned is at an end. Growth of cells subsequent to division may, and generally does, in his opinion, distort the relations of the cleavage planes.

Sachs makes no attempt to include the multinucleated sporangia in his discussion. Still he specifies the growth of the *Siphonocladus* (19, p. 100) as exceptional when compared with the irregular growth of many thallophytes, and considers the development at their growing points as typical of that in the higher



plants. He also specifically states that typical mechanomorphoses are the cell nets in growing points of young organs *and structures whose cells show no individual growth after cell division is complete*. All the sporanges mentioned above would be included in this latter type of structures. The sporange of *Synchitrium* is a spherical mass of protoplasm which divides into cells which show no further growth, at least till after cleavage is complete. At this stage, then, the cleavage planes should illustrate typical mechanomorphosis. It would seem that in such a spherical mass of undifferentiated protoplasm the opportunity for the law of rectangular intersection to come to full expression would be especially good. We might expect the periclinal and anticlinal cleavage planes to be extremely conspicuous in such a case. On the contrary, as noted above, and as is well shown in *figs. 2, 4, 14, 15, pl. 24*, of my former paper (4), no regular periclinal and anticlinal planes are to be observed. The surface furrows cut into the mass at very varying angles, frequently also becoming curved and branching so as to intersect near the surface, and thus cut off superficial cell-masses of the most irregular shapes and sizes. There is apparently the greatest irregularity in the orientation of the cleavage planes both with reference to each other and to the surface of the dividing mass, as a glance at the figures referred to above will show, and as I have described more fully in the case of *Fuligo*. As this method of progressive cleavage, however, is of wide occurrence among the thallophytes, the principle of rectangular intersection loses that universal validity for cell division upon which Sachs so strongly insisted. Sachs uses the law of rectangular intersection so as to further support Hofmeister's doctrine of the subordination of cell division to the growth of organisms as wholes, and in this sense his results have, for the most part, been taken up and utilized for theoretical purposes in discussions on the nature of the cell and its division (see 6 and 26). With Hofmeister, Sachs considers that the growing point of such a structure as the pine shoot develops simply as a mass of protoplasm analogous to that at the growing end of a *Vaucheria* filament. The shape of the end

of the shoot has been determined by selection during phylogenetic development; its growth is the enlargement of a protoplasmic mass along lines predetermined. The putting in of the cell walls, being a purely mechanical process following the simple rule of rectangular intersection, can in no way be regarded as determining the shape of the shoot. The tip grows just as does the *Vaucheria* tip, the formation of individual cells being a purely secondary matter. The independence of the single cells, their growth and division, is entirely subordinated to the growth of the entire organ. It is an open question whether the rectangular intersection of cell walls in vegetative points, assuming that the facts are as Sachs describes them, is sufficient basis for so important a conclusion as that above stated.

Jennings has characterized the law of rectangular intersection as "hardly to be considered as more than a statement of a condition commonly found." There is nothing inherently impossible in the assumption that the habit of forming successive cell plates so that they intersect at right angles has itself been acquired by the cells as individual units. The form of the shoot may then depend on this power of the cells, assuming always a further regulation of their activities by reciprocal stimuli between the cells themselves as well as by stimuli from their environment.

There can be no question that at least the details of cleavage in the sporanges I have studied have been modified in the course of phylogenetic development with especial reference to the needs of the organisms concerned. I have elsewhere pointed out the correlation between the abbreviated cleavage process in *Sporodinia* and its more rapid spore development. The participation of vacuoles in the formation of the cleavage furrows in *Pilobolus* is another example of such modifications. That the cleavage processes are after all so similar in the different sporanges studied is doubtless due to the fundamental physical and chemical properties of the protoplasm, but the process is none the less to be conceived of as modified by selection. It

must also be borne in mind constantly that in such cleavage phenomena as these the cleavage planes are influenced in no way by the need of forming any specific tissue or plant form. In the growing point of a metaphyte it may perhaps be difficult to decide whether the planes of division are determined by the cells themselves in accordance with internal conditions, or whether, as Sachs claims, it is the shape and differentiation of the shoot taken as a whole which determines the planes of division. In the division of these spore-forming masses no such question can arise, since no differentiated tissue is to be formed. The problem is simply to divide the large mass into smaller masses more convenient for distribution. In doing this, as I have shown, entire irregularity prevails as to the orientation of the cleavage planes with reference to each other, and with reference to the axes of the mass to be divided. It may be concluded that the protoplasm is *per se* perfectly isotropic so far as cleavage is concerned, and that it is a matter of indifference whether the cleavage planes intersect at right angles. If rectangular intersection is the rule in the higher plants, it might well be argued that this is a secondarily acquired condition, assuming with Pfeffer that these multinucleated structures are single cells.

The question can hardly be raised in this connection whether the organism or the cell forms the new cells or spores. Still it is interesting and significant to note, as I have pointed out already, that the process of cleavage in these sporanges is essentially similar in principle to the typical cell division by constriction, which I have described as producing the one-nucleated cells at the base of the conidiophore in the mildews, and which is also found in *Cladophora*. Progressive cleavage by surface furrows is only a more complex modification of cell division by constriction. Spore formation is simply cell division, though not by repeated bipartitions of the mother cell, as it commonly occurs in the vegetative growth of the higher plants.

The growth of the *Multinucleatae* cannot then be regarded as illustrating the growth of an organism comparable morphologically to a metaphyte but without cell formation. The

cœnocyte is a cell, and its growth and differentiation is comparable to the growth and differentiation of a single cell in the growing point of one of the higher plants. That such a cell may become multinucleated is illustrated by the multinucleated cells in the red algæ and the striped muscle fibers of animals. Just as in these cases, the cœnocyte everywhere is a cell which has become multinucleated strictly for functional purposes requiring the distribution of the nuclear material through the enlarged cell body.

The more modern theories as to the division of the animal cell all assume a definite correlation between nuclear and cell division. The mechanism of the one is definitely connected with that of the other. The division of the cell at right angles to the long axis of the karyokinetic spindle is the rule among the higher animals as in the higher plants, and the later theories have assumed this relation as fundamental. Thus, Heidenhain's (5) theory assumes that division of the cell is a result of tensions in the unequally stretched elements of a "system of organic rays" extending from the centrosome to the plasma membrane. Kostanecki (11) attributes cell division to the development of a cell plate which is formed as a result of the migration of the ends of pairs of polar rays, produced by the splitting of parent rays, from the points of their original attachment to the plasma membrane of the mother cell into the equatorial region between the daughter nuclei. Jennings (10) has summarized the theories of cell division and of the orientation of the successive cleavage planes in animal cells, and I need not enumerate them here. They are for the most part directed especially to the explanation of cases where nuclear and cell division have become definitely correlated, and in many cases they assume the determination of the plane of cell division by the axis of the nuclear spindle. Cases of cell division between resting nuclei, such as are found in the cleavage of the insect egg, have received less attention. Still, in all the sporanges studied it is plain beyond all else that nuclear division neither determines nor is in any way connected with cell division, and it is thus shown that the

cytoplasm can divide without any reference to the division of the nuclei. The cytoplasm both initiates and completes the process of dividing any particular cell mass, while the nuclei are throughout in the resting condition in *Synchitrium*; and, on the other hand, in *Fuligo* it can carry on the cleavage in essentially the same fashion simultaneously with nuclear division, without apparently being influenced in the least degree by the occurrence of the latter. In *Fuligo* the axes of the nuclear spindles and the planes of the cleavage furrows may be inclined at all imaginable angles to each other (*figs.* 5-7, 8, 9). It is always to be noted in these cases that the cleavage furrow in question is not the one destined to separate the daughter nuclei which are in process of formation at the time when the furrow is forming.

The cleavage always lags behind nuclear division, never separating any two daughter nuclei until they have reached at least the resting condition, or are themselves engaged in the next following nuclear division. This latter case, as found in *Fuligo*, is especially interesting as showing most clearly that the apparatus of nuclear and cell division is entirely distinct. The mechanism of nuclear division is in full operation while cleavage furrows are also forming in adjacent regions of the cytoplasm in entire independence of it. It is thus shown that no such conditions exist here as in endosperm formation in the lilies, where, after nuclear division is complete, a set of new connecting spindles are formed between the resting daughter cells. The mechanism of cell division in the endosperm is the same as in ordinary cell division, which follows at once upon nuclear division. It is presumptively the mechanism of nuclear division which is in operation in this simultaneous cell division, though operating independently of and subsequently to the completion of a long series of nuclear divisions. In endosperm formation we find no cases in which the nuclei are themselves dividing at the same time that cell plates are forming between them and adjacent nuclei which are also dividing. On the contrary, I have observed considerable evidence in the division of the pollen mother cells of the larch, in cases where the first nuclear division is not to be followed by

cell division, that the fibers of the connecting spindle are utilized, at least partially, in the formation of the spindles for the second nuclear division.

It is by no means to be argued from the fact that cell division and nuclear division are independent in these simple forms of plant life that the processes are not most intimately connected in the higher plants and animals, nor that the position of the nuclear spindle may not determine absolutely the plane of cleavage in these latter cases. In the higher plants there can be no doubt that the spindle, persisting after nuclear division, forms a cell plate and determines the plane of cleavage. On the other hand, the process of cell division in *Fuligo* shows very clearly that such a correlation is by no means fundamental or universal. Whether or not the nucleus in any fashion influences the orientation of the cleavage planes in this latter case, it does not do it by means of the karyokinetic spindle.

Wille (25) in a preliminary communication has reported the discovery of a type of division in multinucleated cells in which the nuclei participate in the formation of new cross walls. Just what the nature of the nuclear activity is in this case is not clear from the brief report referred to. It certainly represents a new and most interesting condition in multinucleated cells.

It is quite possible that the irregular cleavage of the fungus sporangia indicates a primitive condition when nuclear and cell division are entirely independent processes, and that the correlation of the two has been gradually achieved in the evolution of the higher plants and animals. It is plain, therefore, that the theories of Heidenhain and Kostanecki (5 and 11) can have no application in the explanation of cleavage in these sporangia, and it is further plain that no theory which interprets cell division as a function of the karyokinetic figure can claim to be of fundamental value for the explanation of the process. Cell division may be much more definitely related to purposeful results in tissue formation when, as in the higher plants, it is accomplished by the mechanism of the spindle and cell plate. It is quite possible that the regular orientation of successive cleavage planes

so as to form tissues, *i. e.*, aggregates of cells with specific shapes and dimensions, only became possible after cell division came to be a function of the same mechanism which effects the division of the nucleus, but, as Strasburger (22) long ago pointed out, the process itself is by no means necessarily dependent upon the existence of this particular mechanism or of any correlation whatever with nuclear division.

Hofmeister (8) calls attention to the fact that the division of protoplasmic masses for the formation of reproductive cells is quite universally accompanied by loss of water and reduction of volume with increased density of the protoplasm. This phenomenon of contraction and loss of water is especially conspicuous in the process of spore formation in sporanges, as I have already noted. It seems a fairly natural assumption that the tensions set up in a mass of protoplasm which is contracting as a result of loss of water may be utilized in some fashion to produce the extremely irregular cleavage furrows which we observe in the early stages of spore formation. That these furrows, however, are not purely mechanical in their origin and analogous to the fissures that appear on the surface of a drying colloidal mass is shown, as I have noted elsewhere, by the fact that, although irregular, they never cut off segments containing no nuclei, and ultimately they produce approximately equal uninucleated spores. Some form of organization must be assumed to be present in the protoplasm which determines the progress of the cleavage so as to lead to a constant result. Still, even with this assumption, the possibility remains that the source of the energy which is thus controlled may be in the tensions produced by contraction due to loss of water.

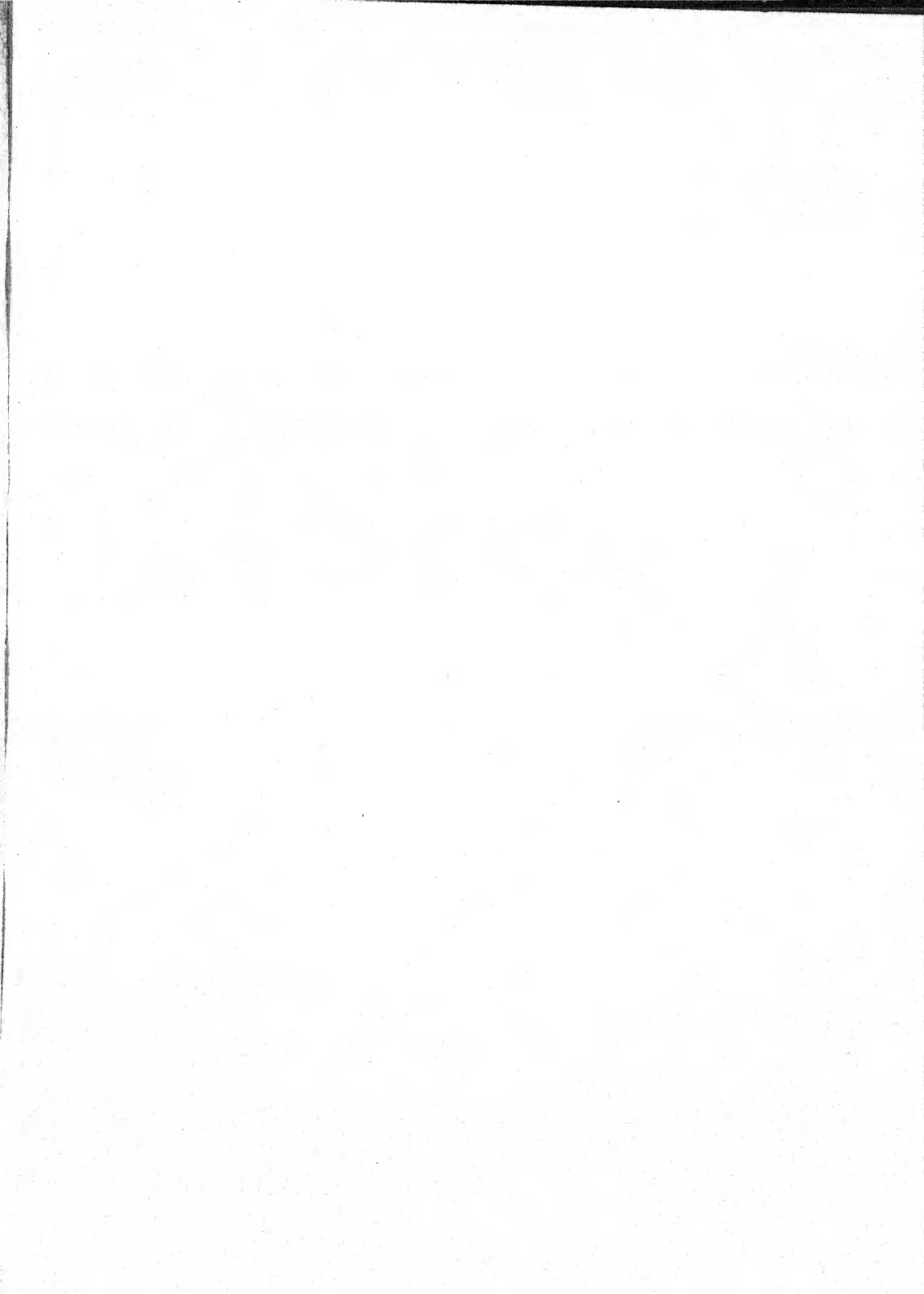
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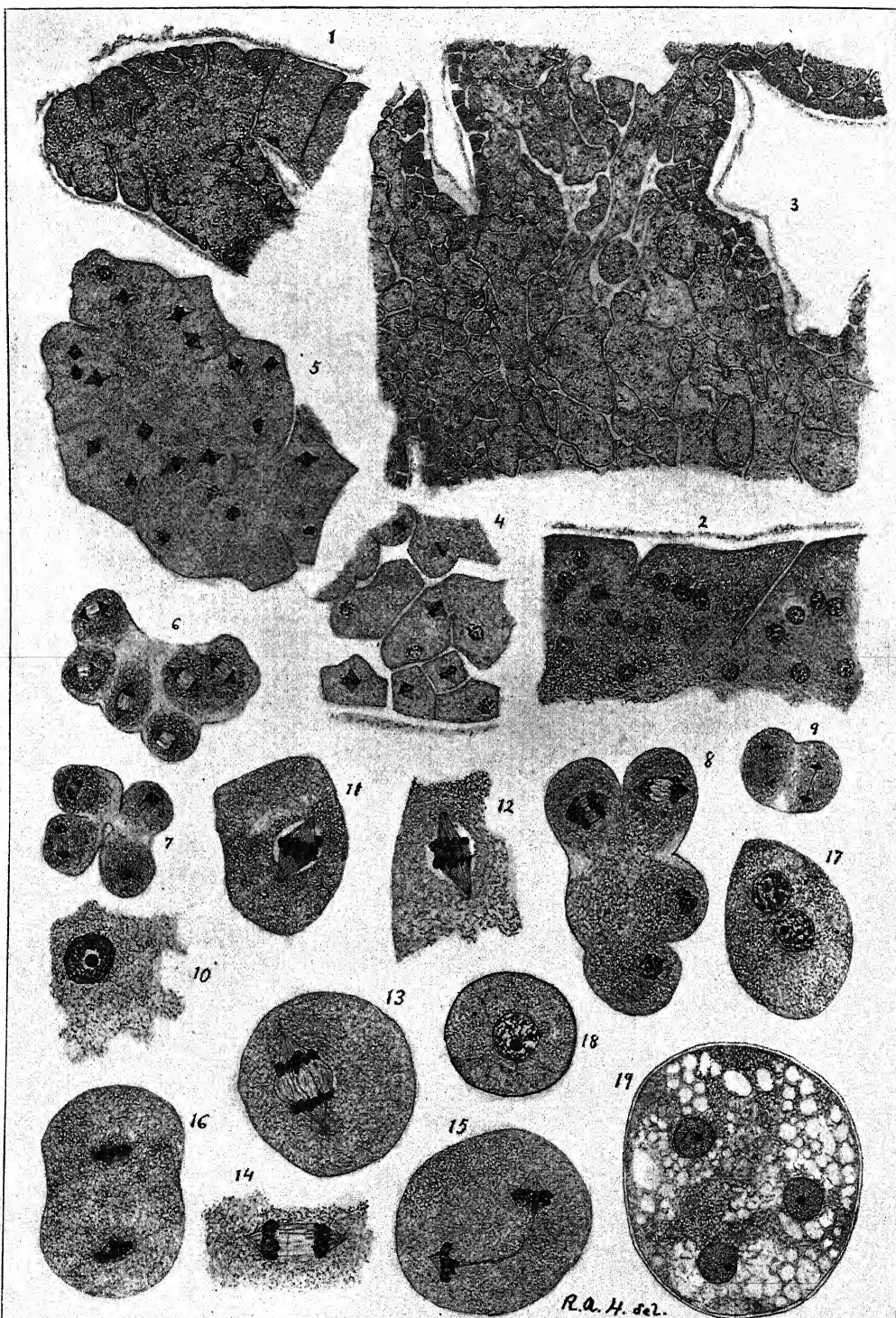
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R. A. H. det.

## EXPLANATION OF PLATE XIV.

All figures were drawn with the aid of the Abbé camera lucida, and all but 1 and 3 with the Zeiss apochr. obj. 2<sup>mm</sup>. Figs. 2-7, and 9 with oc. 6; fig. 8 with oc. 12; and figs. 10-18 with oc. 18.

FIG. 1. Portion of aethalium showing first cleavage furrows and lacunae of the protoplasmic reticulum.  $\times$  about 150.

FIG. 2. Superficial portion of protoplasm showing two cleavage furrows and scattered nuclei.

FIG. 3. Portion of dividing protoplasm from interior of aethalium and lying between three lacunae marked *L*.  $\times$  about 250.

FIG. 4. Several entire segments and portions of others from surface of lacuna in the interior of an aethalium; profile and polar views of nuclei in the equatorial plate stage.

FIG. 5. Segments from interior of aethalium with cleavage furrows cutting into it from various directions; nuclei in equatorial plate stage.

FIG. 6. Segment cut through by hyaline areas which predetermine the course of the cleavage furrows.

FIG. 7. Later stage in cleavage of a segment like that shown in fig. 6.

FIG. 8. Segment showing cleavage furrows cutting through undifferentiated protoplasm and others cutting into hyaline areas.

FIG. 9. Binucleated segment, the nuclei in a late stage of division and separated by a hyaline zone.

FIG. 10. Resting nucleus.

FIG. 11. Uninucleated segment; nucleus in equatorial plate stage; nucleole and part of nuclear membrane of parent nucleus still present.

FIG. 12. Metaphase; part of nuclear membrane of parent nucleus still present.

FIG. 13. Uninucleated segment; nucleus in later metaphase, connecting fibers curved.

FIG. 14. Later stage; connecting fibers straightened.

FIG. 15. Diaster; connecting fibers a narrow strand.

FIG. 16. Dispirem; cell division beginning.

FIG. 17. Binucleated segment; cell division not yet begun.

FIG. 18. Uninucleated spore with thin wall; granules of reserve material in cytoplasm.

FIG. 19. Encysted segment.

DOUBLE FERTILIZATION IN COMPOSITAE.  
CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY.

XXI.

W. J. G. LAND.

(WITH PLATES XV AND XVI)

IN August 1898, Nawaschin communicated to the Russian Scientific Congress at Kieff the results of his work on fertilization in *Lilium Martagon* and *Fritillaria tenella*. Guignard, upon learning of the discoveries of Nawaschin, contributed to the Academie des Sciences a short account of his unpublished researches upon fertilization in several species of *Lilium*. Miss Sargent, from a reexamination of her preparations of *Lilium Martagon*, fully confirmed the observations of both Nawaschin and Guignard. A recent study of *Tulipa sylvestris* and *T. Celsiana* by Guignard gives results in strict accord with the earlier observations.

These observers find that both male cells upon emerging from the pollen tube are vermiform and twisted on their axes, suggesting the idea of non-ciliated spermatozoids. One male cell, coming in contact with the egg nucleus, retains for a time its vermiform shape, gradually enlarges until it becomes nearly spherical, and finally fuses with the egg nucleus. The other male cell fuses with the upper polar nucleus, and the nucleus resulting from this fusion unites with the lower polar nucleus. Sometimes the polar nuclei fuse and then unite with the male cell, and sometimes the polar nuclei and the male cell fuse simultaneously. Preparations of *Lilium Philadelphicum* and *L. tigrinum* in this laboratory show the last named condition. Miss Sargent figures one case in which the ends of the male cell are applied to the polar nuclei, uniting them as if by a bridge. Experiments in hybridization by De Vries and Correns seem to

indicate that double fertilization may be more frequent than is commonly supposed.

So far as recent work indicates, the spermatophytes produce two male cells. The persistent appearance of a second male cell, seemingly as well organized as the one which functions, has found no better explanation than a phylogenetic one, although it would be hard to explain why a cell which has long been abandoned continues to be well organized. We may well inquire whether a simultaneous fertilization of the egg and of the endo sperm nucleus may not be universal in angiosperms.

The study of the mature embryo-sac of *Erigeron* and of *Silphium* was undertaken for the purpose of determining the fate of the second male cell. The first named genus was chosen because a large number of ovules in different stages of development could be cut at once; the second, because of the differentiation shown by the disk and ray flowers.

#### MATERIAL AND METHODS.

Material was collected in the vicinity of Chicago, from June 25 to July 20, 1900. Collections were made at all hours of the day, and all material was killed in the field. The outer involucral scales of *Erigeron* were removed and the heads closely trimmed, only enough of the receptacle being left to hold the ovules together. A 1 per cent. aqueous solution of chromo-acetic acid was used as a killing and fixing agent. Carnoy's fluid was found to be unsatisfactory. The material was passed through xylol into paraffin and cut in serial sections  $3.3\ \mu$  and  $6.6\ \mu$  thick.

The method of treating *Silphium* was slightly different from that for *Erigeron*. The ovules, except those intended for tracing the path of the pollen tube, were freed from the surrounding tissues and immediately plunged into chromo-acetic acid at a temperature of about  $100^{\circ}$ , and were allowed to remain in the hot acid about two hours. After washing and dehydrating, they were passed through xylol into paraffin at a temperature of  $63^{\circ}$ . Sections  $2-5\ \mu$  thick were cut on a Reichert rocking microtome.

Flemming's safranin-gentian-violet-orange G and Haidenhain's iron-alum-haematoxylin both gave excellent results, but the most satisfactory differentiation in fertilization stages was obtained with cyanin and erythrosin. This last combination, after the sections had been treated with acetic acid and chloroform, gave details of structure not obtained by other methods.

#### ERIGERON.

Two species, *E. Philadelphicus* L. and *E. strigosus* Muhl., were examined. The following statements apply to both, as there is no difference in their embryo sacs, except in size. The development of the embryo sac does not differ essentially from that of other Compositae. The number of antipodal cells varies, and the one nearest the endosperm nucleus is frequently binucleate.

The lower polar nucleus moves up and unites with the upper polar nucleus (*fig. 1*), which remains near the egg. The nucleoli are very conspicuous, with highly refractive bodies scattered through them.

The mature egg is pear shaped, its nucleus being very large (*fig. 2*) and showing a fine network of chromatin. A vacuole near the middle of the egg is very conspicuous.

The synergids are pear shaped, and the position of their nuclei is variable. The end nearest the egg invariably has a large vacuole, while the smaller end stains deeply and sometimes has a distinct constriction (*fig. 2*) between the nucleus and the tip.

The embryo sacs in the ovules upon the edge of the head are larger than those at the center, several cases being observed in which the sacs in the center of the head did not function. In *E. Philadelphicus* the usual length of the embryo sac when ready for fertilization is about  $216\ \mu$ , the width being about  $40\ \mu$ . The endosperm nucleus at this stage is from  $14.4$  to  $19.8\ \mu$  in diameter, its nucleolus being usually  $9\ \mu$ . The egg nucleus seldom varies from  $10.8\ \mu$ , with a nucleolus measuring  $5.4\ \mu$ . The endosperm nucleus is usually about  $16\ \mu$  distant from the egg. Chamberlain has shown that in *Aster Novae-Angliae* the nucleolus

of the endosperm nucleus is very constant in size. Many measurements of the egg nucleolus of *E. Philadelphicus* were made, and in only one instance did it vary from  $5.4 \mu$ .

The male cells were not observed in the microspores, and no pollen tubes were found except in the embryo sac. When the pollen tube (*pt*, *fig. 3*) enters the sac, the synergids rapidly disintegrate, so that by the time fertilization is effected only fragments of their nuclei are visible. One male cell fuses with the egg nucleus, and at the time of fusion (*fig. 3*) cannot be distinguished from the egg nucleus. The other male cell fuses with the nucleus which is formed by the union of the polar nuclei, the product of this second fusion being the definitive or endosperm nucleus.

No male cells were observed in the pollen tube, or in the embryo sac before fertilization, so that nothing can be said concerning their previous appearance. The pollen tube, after it has discharged the male cells, usually contains two bodies of irregular shape (*fig. 3*), which stain intensely with cyanin. They were also observed in preparations of *Lilium*. These bodies may be mistaken easily for male cells, especially after fusion is complete. Nothing could be determined concerning their origin, although it is possible that they may have come from a division of the tube nucleus, since that still remains to be accounted for.

After a brief rest the definitive nucleus divides, and in the many preparations examined the cell plate was invariably parallel to the longer axis of the sac. The endosperm nuclei, after the last named division, are usually multi-nucleolate. The fertilized egg in the meantime shows little change, except a thickening of the wall and a slight enlargement; also it moves down nearer the center of the sac. At this stage scarcely any traces of the synergids can be seen.

In the second division of the endosperm nuclei the cell plate is usually at right angles to the long axis of the sac. The two upper nuclei resulting from this last division move towards the micropylar end of the sac, and, occupying the place made vacant by the synergids, lie a little above and close against the

egg. This last position may have been taken because of a movement of the nuclei in the direction of least resistance, which would be towards the place lately occupied by the synergids.

The fertilized egg usually completes its first division shortly after the second division of the endosperm, the first wall being transverse. The further development of the embryo does not differ from that of the other Compositae which have been investigated.

#### SILPHIUM.

*Silphium integrifolium* Michx., *S. terebinthinaceum* L., and *S. laciniatum* L. were studied. The last named species received more attention than the others on account of the large size of the ovules, and the ease with which they could be oriented for sectioning. Merrill's recent paper on the life history of the various species is so complete, except in regard to fertilization, that we will pass over all other stages.

As in *Erigeron*, the polar nuclei fuse long before fertilization, and the resulting nucleus comes to rest near the egg. A careful study of the microspores of *S. laciniatum* revealed the presence of long male cells, lying side by side and reaching almost around the interior of the spore. The pollen tube usually enters at one side of the synergids. In one preparation it was observed to turn almost at right angles, pass between the nucellar cap and the synergid, and then continue its course towards the egg. The synergid against which the pollen tube lies soon begins to show signs of disintegration, the other usually remaining intact until the fertilized egg begins to divide. In all preparations examined the pollen tube was much expanded above the nucellar cap, and its walls stained intensely.

*Fig. 7* shows two small bodies (*x*) similar to those noted in *Erigeron*, which remain in the pollen tube after the male cells have been discharged. This figure also shows the coiled male cell (*sp*<sub>1</sub>) resting against the egg nucleus (*o*). The second male cell (*sp*<sub>2</sub>) is lying against the endosperm nucleus (*e*). The path by which it reached its destination can be seen



clearly. The cause of the peculiar appearance of the endosperm nucleolus shown in *fig. 7* is not known, but it is not believed to be due to reagents, as the other structures appear to be normal. *Fig. 8* also shows the coiled male cells lying against the egg and the endosperm nucleus. *Fig. 9* shows a more advanced stage of fertilization, the male cell fusing with the egg (*a*), having lost its coiled form. In the other (*b*), a trace of the coil may still be seen faintly. One is closely applied to the egg nucleus, the other to the endosperm nucleus. The irregular shape of the endosperm nucleus shown in *fig. 7* is also apparent here. Nawaschin, in a paper received since the above mentioned figures were drawn, describes coiled male cells in the embryo sac of *Helianthus annuus* and of *Rudbeckia speciosa*. His description of the reticulated porous structure shows them to be similar to those found in *Silphium*.

The large amount of food present in the egg is shown in *fig. 9*, the deeply stained bodies being starch grains.

After fusion with the male cell the endosperm nucleus divides rapidly (*ee, fig. 10*), and soon the sac is filled with a mass of nuclei. The fertilized egg (*fo, fig. 10*) does not divide immediately after fertilization, but rests for some time. It may be of interest to note that the nucleus of the upper antipodal becomes very large at this stage. The appearance of the antipodals suggests that they, for a time at least, may possibly serve to transmit food to the embryo, and also to the endosperm.

Merrill has shown that eight is the characteristic gametophyte number of chromosomes, and he believes that sixteen is the number in the tapetal cells. The writer was unable to make an accurate count, but sixteen appears to be the number in the embryo, and more than sixteen were counted in the endosperm. It is believed, as the result of many counts, that the number in the endosperm is twenty-four. This is the number we should expect here, for the primary endosperm nucleus results from the fusion of three nuclei, each containing eight chromosomes.

## SUMMARY AND CONCLUSIONS.

In *Erigeron* the pollen tube passes down a short distance into the sac and discharges the male cells, which bore their way through the surrounding cytoplasm. One male cell fuses with the egg nucleus, the other with the endosperm nucleus. The endosperm nucleus, after fusion with the male cell, rapidly divides and soon fills the sac with a mass of nuclei. The fertilized egg remains some time in the resting condition, doing little beyond developing a dense membrane and becoming slightly larger. The first wall is transverse, as is usual in angiosperms.

In *Silphium* the polar nuclei fuse long before fertilization. The pollen tube passes down into the sac and discharges two male cells. These cells, in some instances, leave a well-marked path through the cytoplasm. They retain their coiled form for some time after contact with the nuclei with which they ultimately fuse. One fuses with the egg nucleus, the other with the endosperm nucleus. Before fusion is completed they become nearly spherical, being slightly flattened on one side.

I may venture to suggest that the fusion of the male cell with the endosperm nucleus is a true fertilization, and not a false one ("une sorte de pseudo-fecondation"), as regarded by Guignard. May not the two nuclei—egg nucleus and polar nucleus—nearest the middle of the embryo sac both be considered egg nuclei; and may not one of them, by some means not yet understood, have an advantage over the other, and so develop a more definite structure? May it not be possible that the fusion of three cells—the polar nuclei and the second male cell—to form the endosperm nucleus imparts to that nucleus an excessive stimulus to cell division, resulting in the rapid cell multiplication which immediately follows fusion?

That both the fertilized egg and the endosperm nucleus are potential sporophytes seems a reasonable view, yet we are confronted with the fact of the fusion of the polar nuclei, and until the meaning of this is made clear we must remain in doubt concerning the true significance of double fertilization.

The spermatozoid form of the male cell may be wide-spread in monocotyls and dicotyls. In all the forms recently examined they have been reported as present, either in the pollen tube, in the embryo sac, or lying against the nuclei. In *Lilium* and *Tulipa* the male cells seem to assume the vermiform shape in the pollen tube, since in the microspore they are spherical. In *Silphium* they assume the elongated form in the microspore.

At present double fertilization is known to be a fact in the Liliaceæ. There are indications that it will be found among the orchids. Among the dicotyls it has been found in *Delphinium elatum*, *Helianthus annuus*, and *Rudbeckia speciosa*. It has also been doubtfully suggested in *Juglans*. The present paper adds to the list *Erigeron Philadelphicus* and *Silphium laciniatum*.

The experiments of De Vries and Correns, and also of Weber, in hybridization of *Zea Mays*, indicate that we may confidently expect double fertilization to be reported soon in that species.

My acknowledgments are due Professor John M. Coulter and to Dr. Charles J. Chamberlain for assistance rendered during the progress of the work.

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#### EXPLANATION OF PLATES XV AND XVI.

All the drawings are made with the aid of a camera lucida, Bausch and Lomb  $\frac{1}{2}$  obj. and ocular 1, giving a magnification of 1260. The plates are reduced to one half the original size. A Bausch and Lomb  $\frac{1}{8}$  obj. was used for studying minute details.

##### PLATE XV. *Erigeron Philadelphicus*.

FIG. 1. Embryo sac with fusing polar nuclei, *e*; synergids, *sy*; egg nucleus, *o*.

FIG. 2. Mature embryo sac showing the long-beaked synergids, *sg*; egg, *o*; endosperm nucleus, *e*.

FIG. 3. Fertilization; pollen tube, *pt*, with two intensely stained bodies, *x*; male cell fusing with the egg, *a*; second male cell fusing with the endosperm nucleus, *b*.

FIG. 4. First division of endosperm.

FIG. 5. Later stage of endosperm division, showing cell plate and multinucleate nuclei.

FIG. 6. First division of fertilized egg.

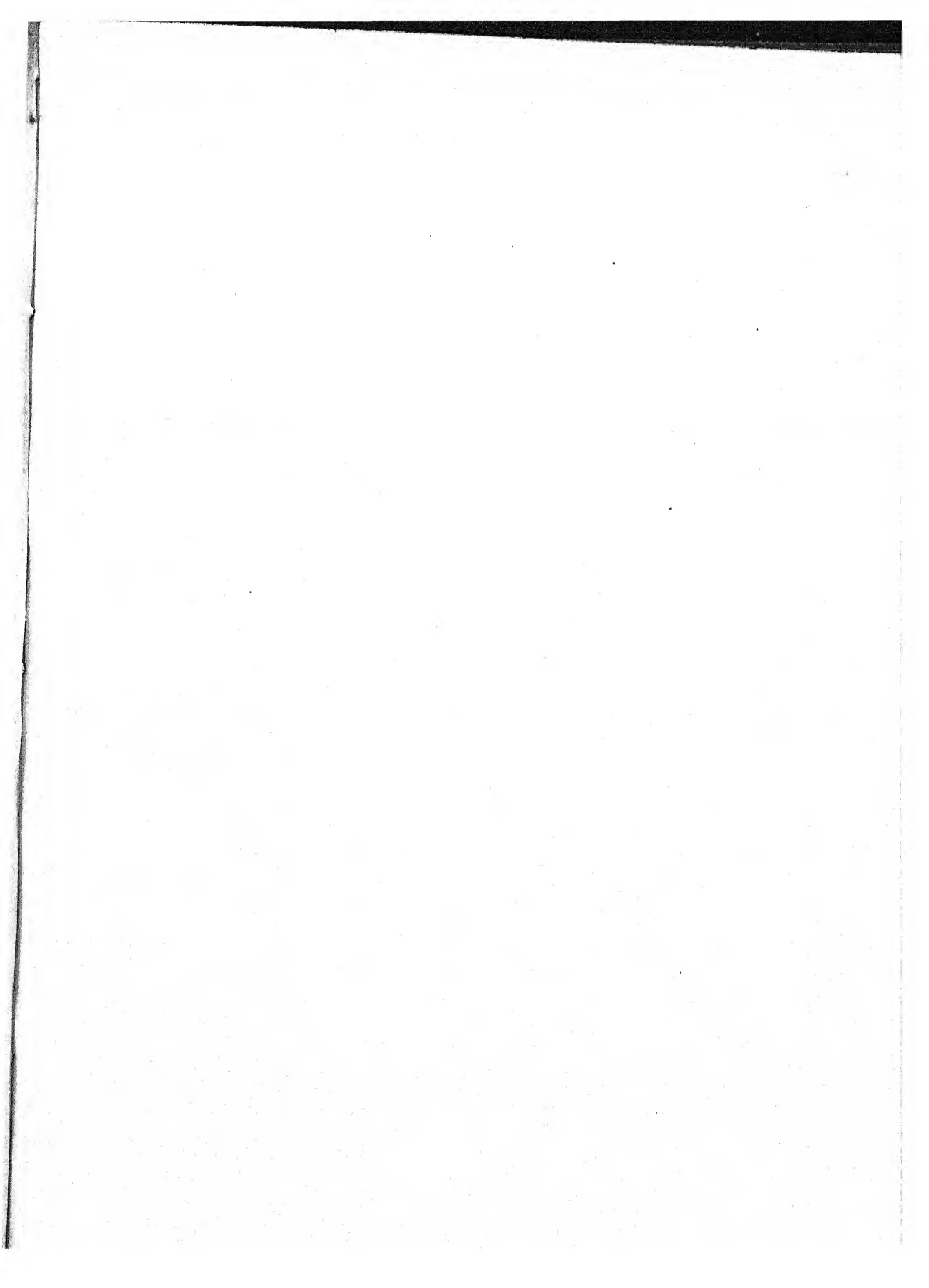
##### PLATE XVI. *Silphium laciniatum*.

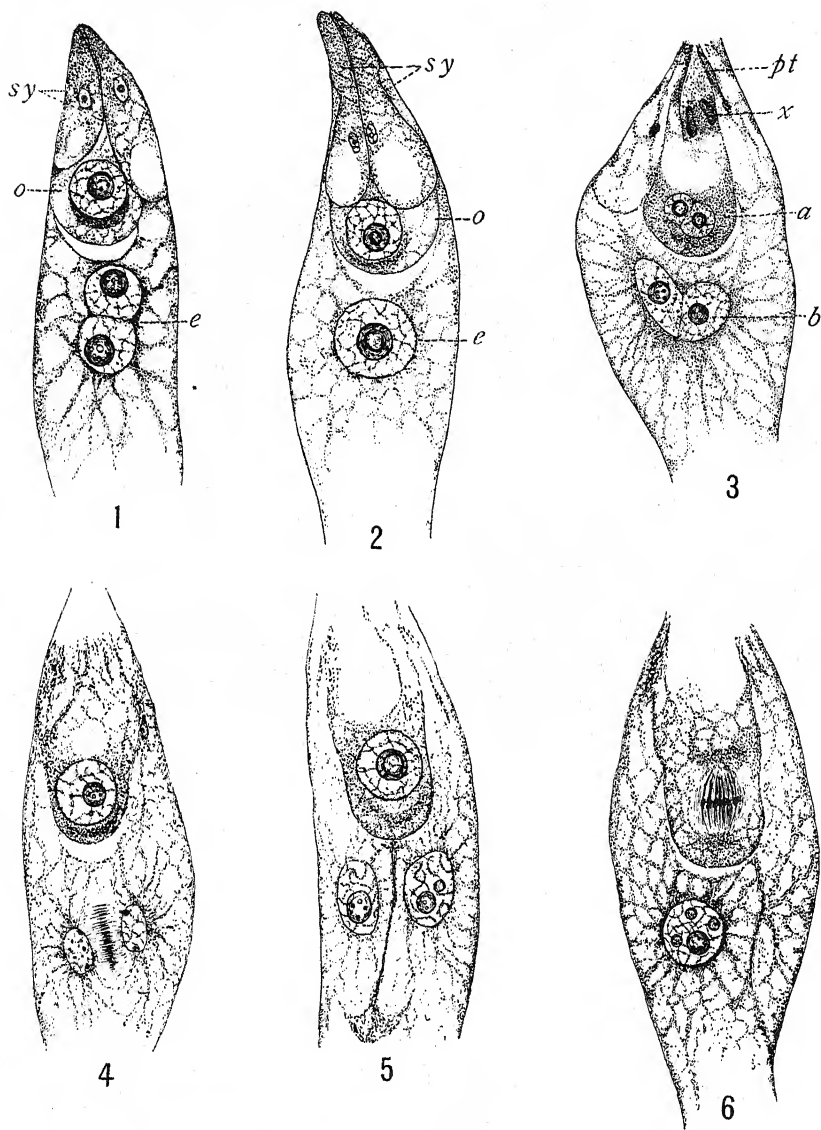
FIG. 7. Fertilization; pollen tube, *pt*, passing into the sac; synergid, *sy*; unknown bodies, *x*; male cell, *sp*<sub>1</sub>, lying against the nucleus of the egg, *o*; male cell, *sp*<sub>2</sub>, lying against the endosperm nucleus, *e*.

FIG. 8. Synergid, *sy*; male cell, *sp*<sub>1</sub>, lying against nucleus, *o* (a portion of this male cell was lost in sectioning); male cell, *sp*<sub>2</sub>, with large coil, resting against endosperm, *e*.

FIG. 9. Later stage of fertilization; pollen tube *pt*; synergid, *sy*; fusion of male cell with egg nucleus, *a*; fusion of male cell with endosperm nucleus, *b*.

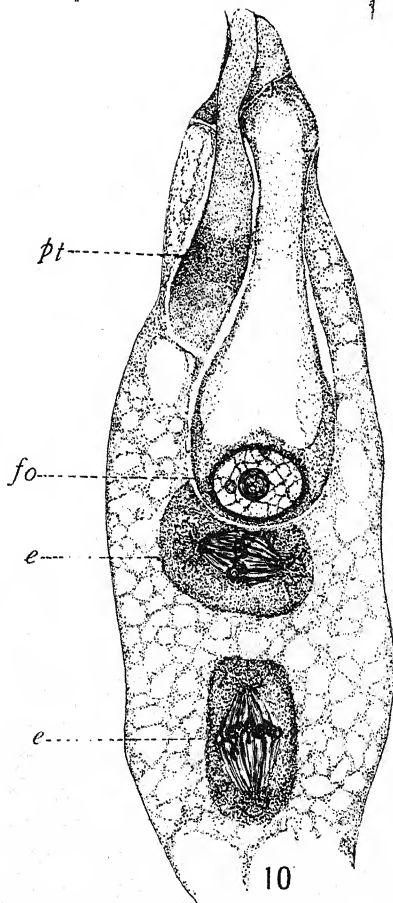
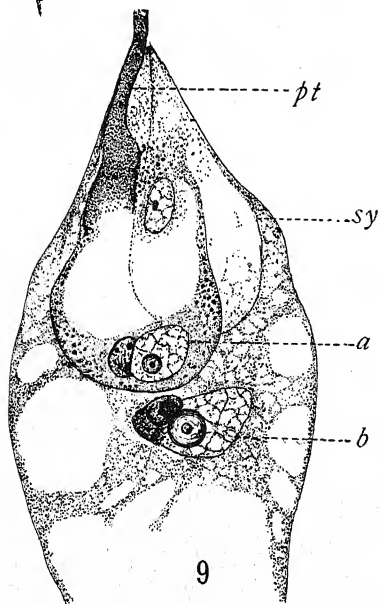
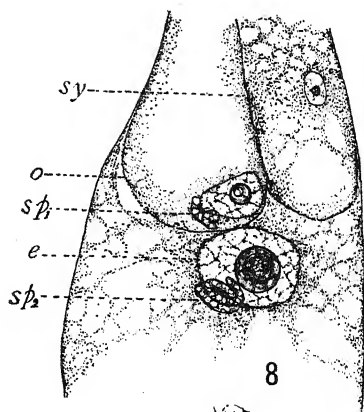
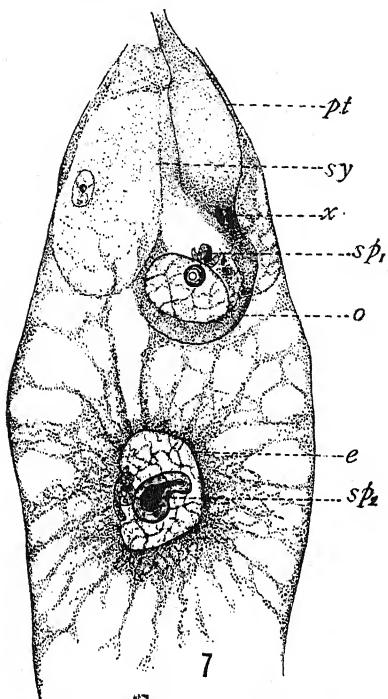
FIG. 10. Second division of endosperm; pollen tube, *pt*; fertilized egg, *fo*; dividing endosperm nuclei, *e*, *e*.





W.J.G.L. del.

LAND on ERIGERON



W.J.G.L. del.





## A NEW CHROMOGENIC MICROCOCCUS.

MARY HEFFERAN.

(WITH FOUR FIGURES)

IN the course of an examination of river water for the Sanitary District of Chicago, carried on during the past year under the direction of Professor Jordan at the University of Chicago, a somewhat unusual opportunity has been afforded for the study of water bacteria. The water for this examination is taken from twenty-seven different stations along the Illinois and Desplaines rivers and their tributaries, and also from the Mississippi river above and below St. Louis. It is collected in sterile bottles with tightly fitting glass stoppers. As soon as the water is collected these bottles are enclosed in small tin cans which are packed with ice in larger cans, and shipped as speedily as possible to Chicago.

In making a qualitative examination of this water it has been plated in ordinary beef peptone agar (Witte's) and in Nährstoff Heyden agar.<sup>1</sup> The latter medium has the advantage of bringing to development a much greater number of colonies than ordinary peptone agar; for instance, at the end of ten days a peptone agar plate and the corresponding one of Nährstoff Heyden may present colonies in a ratio of 21 : 400. The reason for this enormous difference has not as yet been sufficiently determined. It is possible that the large, spreading, and rapidly growing colonies may inhibit the development of the more tardy forms on a peptone agar plate. On the Nährstoff Heyden plate these large colonies, particularly the fluorescent forms, are not seen, or at least appear only as small dots; while on the other hand, the plate is crowded with small, distinct, slowly developing colonies, some of which seem to present novel characteristics. It is likely, therefore, that this medium will furnish varieties not obtained on peptone media.

<sup>1</sup>Hesse and Niedner, *Zeitschr. f. Hygiene* 19 : 460.

Among the germs found in this way, I isolated several times during the course of the winter a micrococcus producing a salmon-pink pigment, which seemed not to have been previously described. This form first appeared in Mississippi river water, collected at "The Chain of Rocks," near the St. Louis water tower. The water in a dilution of 1:100<sup>cc</sup> was plated in 15 acid gelatine and peptone agar, and in neutral Nährstoff Heyden agar, using 1<sup>cc</sup> of the diluted water to each plate. The micrococcus developed only on the Nährstoff Heyden plate, as it did in other experiments with the same water, although on one occasion I found it upon an ordinary agar plate, showing that the special medium is not essential. It also develops readily upon agar in pure culture. Water from other stations along the rivers often gave rise to pink colonies on peptone agar or Nährstoff Heyden, which by casual observation could not be distinguished from those of the micrococcus. A closer examination, however, usually revealed in these cases a bacillus whose pigment upon cultivation showed less of a yellow tinge in the rose than that of the salmon-pink form. On one or two occasions these small pink colonies were found to be due to a yeast, but the bacillus was by far the more common form. The micrococcus may exist in other parts of the rivers, although I did not observe it in any other water than that of the Mississippi. My search for it was only incidental in connection with other work, and the notes upon its appearance refer it always to the locality mentioned.

In the two cases in which the germ was isolated and cultivated through all the stages, it occurred in close connection with a bacillus producing a yellow pigment. The first transfer from a rather crowded Nährstoff Heyden plate or from the more isolated colony upon the peptone agar plate resulted in a mixed culture of the two kinds, a second plating being necessary in order to separate them. The bacillus was not identified. The micrococcus, which it is the purpose of this paper to describe, grows well and is easily cultivated at room temperature in broth

and milk and on all ordinary solid media except potato. The cultural features are as follows:<sup>2</sup>

*Gelatine.* — On a gelatine plate at 15°–20° C. it grows slowly. The colonies were visible on the third day and appeared under low power as small irregular dots, transparent, but with a slight, thin, uniform granulation. On the next day those that had emerged upon the surface had lost their irregularity and had become perfectly circular and slightly larger; otherwise to the eye they were unchanged, no pigment being visible. Under a higher power the granulation appeared more distinct, the colonies seemed thicker and had a yellowish tinge in the center, and were thinner and more transparent at the edge. A few days later the surface colonies had taken on the characteristic salmon tinge. Under the low power they looked perfectly homogeneous except for thinness at the periphery where the fine granulation showed more distinctly. The edges were smooth and clearly defined. Later only a very slow increase in size took place, the colonies reaching a few millimeters in diameter in several weeks.

Gelatine streak culture at 15°–20° C. showed growth on the third day in two delicate, finely wrinkled lines, one each side of the needle track. This was at first a pale creamy-pink in color. On the sixth day the lines were somewhat broader and had lost their delicate wrinkled appearance, the lower parts being confluent and smooth. Later the growth took on the true deep salmon color. There seemed to be almost no softening of the gelatine, except possibly a slight sinking of the line of growth.

Gelatine stab culture at room temperature showed a small cream colored colony on the surface and a slight development along the needle track at the end of the third day. The growth is very slow, the drawing (*fig. 1*) being taken from a three weeks old culture. The colony on the surface increases gradually

<sup>2</sup> Except where otherwise indicated the media used were prepared in accordance with the recommendations of the Bacteriological Committee. Jour. Amer. Pub. Health Assoc., January 1898.

in diameter and becomes more deeply colored, sometimes showing a slight zoning, the deeper pink in the center. The only evidence of liquefaction at first is that the surface of the gelatine sinks perhaps more rapidly than could be accounted for by ordinary evaporation, forming a hollow cup-shaped cavity lined by the smooth dry pink layer of the expanded surface colony. If the gelatine is rather stiff this surface sinking takes place very slowly. This dry cavity may reach two or three centimeters in depth in the course of a month (fig. 2). The deep growth along the needle track remains colorless and little developed.



FIG. 1.



FIG. 2.

*Agar.*—To the eye the agar plate colonies do not differ materially from those on gelatine.

They appear on the fourth day at room temperature as minute dots, which take on a yellowish tinge. Examined under low power they are seen to be very irregular, and unlike the gelatine colonies they do not become smooth edged and round upon reaching the surface, but retain their irregularity, the edges showing a slight raggedness. The texture is uniform and very finely granulated, being somewhat more dense in the center and thinner on the periphery. They become salmon-pink in the course of a few days and increase very slowly in size (fig. 3).

Agar streak shows beginning of development in twenty-four hours as a slight whitish growth along the needle track. In forty-eight hours it has become a light pink, a much more creamy or yellowish-pink than the rose culture of *M. agilis* of a day or two older but of about the same development. Later the growth becomes quite luxuriant, gradually



FIG. 3.

deepening in tint. It has much the same appearance as the agar growth of a pink yeast, *Torula* III, except perhaps for a more decided salmon color. On fresh, moist agar the edges are smooth and the whole growth a straight, shining,

moist layer. If the agar is older the edges are likely to be irregular or lobulated.

The reaction of the agar, ranging 15 acid, neutral, or 15 alkaline, Fuller's scale, produces little perceptible effect, although the early development may be slightly more rapid on neutral agar.

At 37° the growth is very limited, and there is no pigment, but growth proceeds normally on removal from the incubator. Light seemed to have little influence upon pigment production; cultures standing on a shelf near a north window where no sunlight entered had about the same appearance as those kept continually in a dark locker. Development on glycerine and 2 per cent. glucose agar showed no variations from ordinary growth.

*Potato.*—The micrococcus refused to develop on either acid or alkaline potato at room temperature or in the incubator.

*Milk.*—Cultures at 20° C. showed no change beyond a deposit and a thick surface ring of the same characteristic color as the agar and gelatine cultures. Lactose litmus milk brought out this color very well, the litmus itself turning a more vivid blue, indicating an alkaline reaction. In course of a month or more, the liquid showed traces of peptonization without coagulation.

*Bouillon.*—Became cloudy in forty-eight hours and remained so with no change beyond the slow accumulation of a salmon-pink deposit. No production of indol nor reduction of nitrate.

*Fermentation tube.*—Dextrose 2 per cent. showed slow growth in the bulb and accumulation of pink sediment. No cloudiness in the closed arm and no gas.

*Staining reactions, morphology, etc.*—The micrococcus stains readily with ordinary stains and is decolorized by Gram's method. Mounted preparations reveal small cocci measuring about 0.6  $\mu$  in diameter, grouped irregularly with no particular indication of sarcina, diplo- or strepto-coccus form. The unstained specimens in hanging drop showed no motility other than the Brownian vibration. They measure less than 1  $\mu$ , somewhat smaller than unstained specimens of *M. agilis*. No spores observed.

Upon cultivation in gelatine hanging drop under  $\frac{1}{12}$  immersion lens, the cell multiplication appeared as shown in *fig. 4*. I was unable with this power to see the actual process of division, but there was little doubt that it took place in only two planes giving rise to a typical staphylococcus grouping. That division

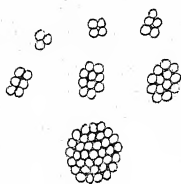


FIG. 4.

did not occur in three directions was evident from cultures in liquid gelatine or broth, in which the cells divided regularly into groups of two cells each, which remained together a short time, then separated into free single cocci. The best series observed was that figured in the drawing, the development occurring in a solid gelatine drop as follows: twenty-four hours after sowing, in which time multiplication is well begun, a group of three cells was brought into focus, carefully examined, and drawn at 11:30 A. M. I examined the group at intervals of a few minutes without seeing any signs of division, but at 2:30 it was evident that two cells had taken the place of one in the group. At 4:00 P. M. there were five cells, and two hours later (6:00 P. M.) an oblong group of six cells could be seen in one place. At 8:00 the next morning ten cells were clearly visible, and the group had begun to acquire the circular form characteristic of all the more advanced colonies in gelatine culture, although in agar hanging drop, as on an agar plate, the colony form remains irregular. At 2:30 P. M. of the second day of observation, only a slight increase had taken place, fourteen cells being in focus in one plane, forming a rosette-like figure. Afterward the growth was very slow, the last drawing of the colony having been made five days after sowing. All colonies showed the same glomerulus structure.

In the endeavor to identify this micrococcus with the pink varieties already described, I found some unexpected difficulties. First, the liquefaction of gelatine by this form is so slight as to pass almost without remark, making it doubtful in which of Migula's two classes the germ should be placed.<sup>3</sup> It is necessary, therefore, to examine his whole series, especially with

<sup>3</sup>MIGULA: *System der Bakterien* 2: —. 1900.

regard to the two points most evidently characteristic of this form, its pigment coloration, and non-development on potato.

Migula describes eleven non-liquefying micrococci as *Rot wachsend*. Of these, seven may be thrown out, since they are distinctly said to have a seal, or carmine red, or vermilion pigment. Another is "gray on agar, red in milk, and does not grow at 37°." The other three are said to be "flesh red," the exact color of which is doubtful. Of these:

(1) *M. carneus* Zimmerman is described by Zimmerman himself<sup>4</sup> as having a deep flesh red, almost violet tinge, and growth on potato. Lustig describes a form, *Coccus ruber*,<sup>5</sup> found in water by Maschek which is probably identical with this.

(2) *M. sarcinoides* Migula (n. sp.)<sup>6</sup> looks often like sarcina. It is also described as a very large coccus, 1.8–2.5  $\mu$  in diameter. Nothing is said of the potato growth. Gelatine plate colonies reach 5<sup>mm</sup> in diameter. Color on agar is a warm flesh red, more intense along the middle line. Bouillon has a flocculent whitish growth and a red sediment. This description, I think, hardly coincides with that of the salmon-pink form, although much depends upon what is meant by "flesh red."

(3) *M. rubellus* (Migula)<sup>5</sup> is morphologically scarcely distinguishable from the last, and shows the same sarcina grouping. The colonies are almost opaque, dark gray-brown. The description of the gelatine stick culture has some resemblance to that of the salmon-pink form, *i. e.*, a dry surface colony slowly spreading to walls of tube and having underneath a deep dry cavity of non-liquefied gelatine. But this growth is said to be a "bright flesh red." All cultures have a trimethylamine odor; on agar streak the edges are finely dentate, and bouillon has a red slimy sediment, enough points of difference to distinguish the forms.

In the class of germs which Migula describes as liquefying gelatine, we find out of the twelve named that three have carmine red pigment (*M. rubens*, *M. persicus* Kern, *M. haematodes* Babes); one is wine red (*M. rubiginosus* Kern); one has a violet

<sup>4</sup>Die Bakterien unserer Trink- und Nutzwässer. Chemnitz, 1890.

<sup>5</sup>Diagnostik der Bakterien des Wassers 40. 1893.

<sup>6</sup>Op. cit.

tinge (*M. roseo-persicus*); and one (*M. fragilis* Dyar) is "brown-red on glycerine agar." These colors are not likely to be mistaken, and cut the list down to those which are either "rose" or "flesh red." Three of these (1) *M. roseus* Flügge, (2) *M. roseus* Eisenberg, and (3) *M. roseus* Maggiora are sometimes confused because of the similarity of name.

(1) *M. roseus* Flügge is only briefly mentioned by Flügge himself,<sup>7</sup> but is described more at length by Macé<sup>8</sup> as a form common in air, non-liquefying on a gelatine plate, slowly liquefying in a stab culture. But he says that the liquid portion is tinted red "more vermilion than by *prodigiosus*." Macé also mentions among species closely related to this, *M. agilis* Ali-Cohen, *M. rouge cerise* of List, isolated from water and producing a cerise color on potato, and Zimmerman's *M. carneus* which Macé, as well as Lustig, regards as identical with Maschek's *Coccus ruber*. He describes these as all non-motile cocci, about  $0.8\mu$  in diameter, "rose red or flesh colored," and abundant on potato.

(2) *M. roseus* Eisenberg,<sup>9</sup> to which Migula gives the name of *M. subroseus*, is found in sputum and produces a pigment like that of *B. prodigiosus* on potato.

(3) *M. roseus* Maggiora is described by Ward<sup>10</sup> as a non-liquefying form  $0.6\mu$  in diameter, associated in irregular glomeruli, and forming a pale rose pigment. I was unable to gain access to a further description of this micrococcus.

As to the remainder of Migula's list there is left:

(4) *M. cumulatus* Kern, a too definitely liquefying form to be identified with the salmon-pink micrococcus. Otherwise, excepting the fact that it is a facultative anaerobe, the descriptions are somewhat similar.

(5) *M. subcarneus* Migula shows in a gelatine stab culture after one day a small light red colony at point of incision. After three weeks a sirupy liquefaction begins in stocking-shaped

<sup>7</sup> Die Mikroorganismen 185 [3d ed].

<sup>8</sup> Traité pratique de bactériologie 432. 1891.

<sup>9</sup> Bakteriologische Diagnostik 408. 1891.

<sup>10</sup> Annals of Botany 12: 309. 1898.



form, with the colony on the surface and a thick, flocculent, rose-colored sediment. On agar, "rose" or "flesh colored."

(6) *M. rosaceus* (Frankland) is found in air. The entire description is as follows:<sup>11</sup> Cocci which are very variable in size, the largest  $2.5\mu$  in diameter. On gelatine forms a smooth pink expansion on the surface, while needle track below remains almost undeveloped. In older cultures the margin assumes a radiated appearance, while still older cultures frequently exhibit slight liquefaction. On agar, smooth bright pink surface expansion, devoid of any further character. Broth after nine days is clear, free from pellicle, and has a pink deposit. On plate, the colonies appear to the naked eye as pin-head dots on surface, and are bright pink in color. Under low power ( $\times 100$ ) are seen to be of distinctly reddish tint, the edge irregular but smooth. As the colonies approach the surface, the irregularity diminishes.

Certain points in this description agree somewhat with that of the Mississippi river form, *i. e.*, the gelatine culture and plate colonies, but the size of the coccus, the agar and broth culture seem to differ, and the description is too imperfect to make the comparison at all complete. The same may be said of Frankland's description of his (7) *M. carnicolor*, the only stated points of difference from (6) being (*a*) a more rapid growth, (*b*) fainter color of pigment, and (*c*) slightly different appearance of colonies (more circular and brownish-pink). In both these forms the bouillon cultures are said to be clear after nine days, while numerous cultures of the salmon-pink form have remained cloudy for weeks.

This concludes the list of red growing cocci which I have found described, except that mention might be made of Bunn's *Diplococcus roseus* and *Sarcina rosea* (Schroeter), both liquefying air forms;<sup>12</sup> also *Sarcina mobilis* (Maurea),<sup>13</sup> which liquefies and will not develop on potato, but has a brick-red pigment. I have already mentioned *M. agilis* in the body of the description.

<sup>11</sup> Phil. Trans. Roy. Soc. London 178 : 269. 1887.

<sup>12</sup> EISENBERG, op. cit., 12 and 16.

<sup>13</sup> STERNBERG, Manual of Bacteriology 720. 1893.

It is evident that the color of the pigment produced by these germs, otherwise so similar, is of some importance in an effort to distinguish them. The descriptions are, for the most part, very meager in actual definite points, and it is doubtful if even the twenty-six standard tests given by Fuller<sup>14</sup> as showing a high degree of constancy would serve to differentiate these forms, so general in character are the indicated reactions of the table. In regard to the chromogenesis, it would be of no advantage to multiply the list of adjectives, for few bacteriologists could describe in this way the exact tints of "pink," "reddish," "rose," or "flesh color" with sufficient accuracy to make them unmistakable. An article by E. B. Shuttleworth,<sup>15</sup> on "Nomenclature of colors for bacteriologists" is one of the most recent attempts to systematize colors for bacteriology. This author's classification gives under yellowish-pink three subdivisions: salmon, salmon-buff, and flesh color. I was unable, however, to obtain the plates or illustrations to which his numbers evidently referred, and without them the words do not give a very distinct visual image.

A simple and more exact method of determining the pigment color of germs is that of the use of a color wheel. A little contrivance of the kind often used in morphology and easily obtained is a wooden top with the various small color disks. A better determination, because of the more constant rate of revolution of the disks, can be made with a Bradley color wheel and standard Maxwell disks. By this means I obtained the following result in the case of the salmon-pink germ.

An agar culture begins in a cream-pink growth for which the determination is not necessary. After about 5-7 days it begins to acquire the characteristic color, the composition of the color wheel for this age being near: red, 70 per cent.; white, 20 per cent.; yellow, 10 per cent.. Later the color deepens with the addition of a more orange tone, and remains almost constant after two weeks for all culture media—agar (different reactions),

<sup>14</sup> Jour. Expt. Med. 4: 609. 1899.

<sup>15</sup> Jour. Amer. Pub. Health. Assoc. 1895: 406.

gelatine, on which the orange tint is slightly more marked, broth sediment, milk ring. Several trials to match this with the color wheel resulted in a range of:

	(1)	(2)	(3)
Red - - - - -	76%	72%	72%
White - - - - -	10	14	13
Yellow - - - - -	8	9	9
Orange - - - - -	6	5	6
	100	100	100

Of these, (3) was almost the exact tint of my cultures, with the one difference of a slightly duller tone than that shown by the actual growth, particularly of a rather bright gelatine culture. But any one of these combinations gives a good idea as to the characteristic tint.

By this method of description of colors there would be less possibility of confusion. The great advantage would lie in the systematization of those chromogenic germs whose color is fairly constant on ordinary media for certain ages. For instance, the whole *prodigiosus* group produces a color in liquefied gelatine which may be variously described in the vocabulary of different bacteriologists; but the fact that it closely approximates a color combination on the wheel of red 80 per cent., black 20 per cent., is sufficiently definite. *B. prodigiosus* and *B. ruber balticus* both give the above color to liquefied gelatine in a ten-day culture. In addition to this *B. ruber balticus* produces a heavy surface membrane of an entirely different shade, *i. e.*, red 80 per cent., orange 20 per cent.

The one difficulty for this accurate determination of pigment color is, of course, the liability of variation in the pigment produced by the same germ on media made by different bacteriologists or in cultivation under different circumstances. However, it has been found that if this organism is brought into good physical condition by a series of broth and gelatine cultures, according to Fuller's suggestion,<sup>16</sup> such variations in standard media are not of very wide range.

<sup>16</sup> Jour. Expt. Med. 4: 609. 1889.

Because of the peculiar salmon or yellowish-pink color of the pigment produced by the organism described in this paper, and because, although it is evidently closely allied to some of the pink micrococci previously described, it is not identical with any of them, I suggest for it the name *Micrococcus roseus flavus*.

In conclusion, I wish to express my sincere thanks to Professor Edwin O. Jordan for many valuable criticisms and suggestions during the progress of this work.

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## BRIEFER ARTICLES

### A NEW SPECIES OF NEOVOSSIA.

(WITH ONE FIGURE)

DURING September 1899, while collecting near Colo, Iowa, I found that the ovaries of *Phragmites communis* were affected by a peculiar smut. The specimen proved so interesting that Dr. Pammel, to whom I referred the matter, sent material to Dr. Farlow, who replied: "Your spores are perhaps a trifle smaller and have a slightly more marked episporium than in *Neovossia Moliniae*. Still the differences are very slight, and without further study I should not know whether to call them specifically different or not. You may have the material to determine this point." Accordingly Professor Hume, who is working upon the Ustilagineæ of Iowa, after a thorough examination and comparison of the specimens, decided that it was sufficiently distinct for a new species.

This genus was founded and distributed by von Thümen,<sup>1</sup> who called it Vossia. As this name had been given to a genus of grasses, Körnicke<sup>2</sup> changed it to Neovossia. Saccardo,<sup>3</sup> in his treatment of the Ustilagineæ, refers this fungus to *Tilletia Moliniae* (Thüm.) Wint., following Winter.<sup>4</sup> Dietel,<sup>5</sup> in describing the Ustilagineæ and Tilletiineæ, uses the Neovossia of Körnicke. Massee,<sup>6</sup> in his revision of the genus Tilletia, refers it to Neovossia, saying: "This species differs from Tilletia in the mode of spore germination, and must consequently return to Neovossia." Quite recently, Magnus<sup>7</sup> has discussed Neovossia, and considers it separated from Tilletia by the fact, discovered by Brefeld<sup>8</sup>, that in germination the conidia do not copulate. He also considers that von Thümen has well founded the genus upon the fact

<sup>1</sup> Mycotheca universalis 1216. 1878; Oester. Bot. Zeitschr. 29: 18. 1879.

<sup>2</sup> Oester. Bot. Zeitschr. 29: 217. 1879.

<sup>3</sup> Sylloge Fung. 7: 486. 1888.

<sup>4</sup> Rabenh. Krypt. Fl. Pilze 1: 109. 1884.

<sup>5</sup> Ustil. u. Tillet. Nat. Pflanzfam. 1: (Lief. 160). 1897.

<sup>6</sup> Bull. Roy. Gard. Kew 153, 154. 1899.

<sup>7</sup> Ber. d. deut. bot. Gesells. 18: 73. 1900.

<sup>8</sup> Unters. Mykol. 12: 210.

that the ripe spores, with the upper end of the sterigmata, break off from the full grown sterigmata. There seems to be sufficient reason, therefore, for establishing the genus *Neovossia*.

*Neovossia Iowensis* Hume and Hodson, n. sp.—Spore mass filling the ovary, black; spores globose, subglobose, or ovate, brownish-black,

opaque,  $16 \times 20$  to  $24 \times 28 \mu$ , enclosed in a hyaline capsule; appendage slender, hyaline, two or three times the length of the spore; epispore apparently pitted.

A careful comparison with specimen no. 1216 of von Thümen's *Mycotheca universalis* leads to the belief that the Iowa specimens are specifically distinct. The spores differ from those of *Neovossia Molinia* (Thüm.) Körn. in

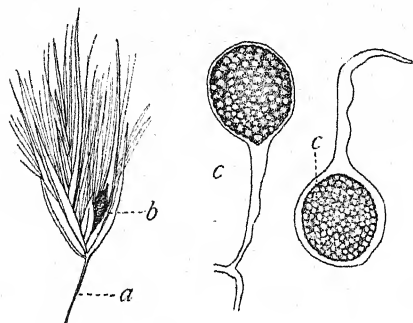


FIG. 1.—*NEOVOSSIA IOWENSIS*; *a*, spikelet of *Phragmites communis*; *b*, affected ovary; *c*, spores.

being darker in color, broader and shorter, and generally blunter at the end opposite the appendage. The markings of the spore, also, are somewhat coarser. Ten spores of von Thümen's specimen, selected at random, gave an average of  $27.7 \times 17 \mu$ , while the spores taken from the material collected at Colo, Iowa, gave  $24.8 \times 18.9 \mu$ .—E. R. HODSON, Ames, Iowa.

#### NOTE ON THE ORIGIN OF TANNIN IN GALLS.<sup>1</sup>

THE origin of the different plant constituents is as much a mystery as their functions, and neither of these questions can be settled until more observations have been made. In considering the origin of tannin in galls the writer limits his observations for the present to the examinations of the common "ink-ball" or "ink-gall," which is produced on *Quercus coccinea* Wang., probably by *Cynips aciculata* O. S. The same kind of gall is produced on other oaks, as Mr. Beadle, of the Biltmore Herbarium, has sent me specimens which were produced on *Quercus imbricaria* Michx.

<sup>1</sup> Presented at the New York Meeting of the American Association for the Advancement of Science, June 1900.

I. These galls are produced during the summer months on the young branches and sometimes on acorns. When mature they fall from the trees and are nearly globular in shape, varying from 20-30<sup>mm</sup> in diameter. They are solid throughout and of the consistency of the pulp of a green apple. Externally they are smooth, and in color are a mottled green, yellow, and brownish-red. At this stage they are made up of three distinct zones: (1) a central zone, made up of nearly isodiametric parenchyma cells packed with numerous small somewhat spherical or irregular starch grains which are colored blue with iodine; (2) the middle zone, composed of radially elongated parenchyma cells, possessing thick cellulose walls with prominent simple pores and containing a mass of protoplasm lying on the sides of the walls and a few starch grains (with the development of the egg of the insect there also appear in the cells of the middle zone numerous starch grains closely resembling those found in the central zone); (3) an external layer made up of irregular parenchyma cells somewhat collenchymatic in character, with the protoplasm as in the middle zone.

II. Decided changes take place soon after the galls fall from the tree: (1) A larva develops in the central zone and there are signs of activity in the protoplasm of the cells of this zone. A large nucleus with nucleolus lies centrally in the protoplasm and in some cases yellowish globular vesicles are apparent. These latter are fixed in the fresh specimens by means of copper acetate (7 per cent. solution), after which treatment they become more yellowish in color and insoluble in water, chloral, glycerin, potassium hydrate, or alcohol, and are no doubt in the nature of tannin vacuoles. (2) In the galls which contain a larva, and have been allowed to remain in copper acetate solution for several weeks or months, there separate in the parenchyma cells of the middle layer or zone yellowish crystals or crystalline masses, which are insoluble in water, chloral, glycerin, or alcohol. These crystals and crystalline bodies are lens-shaped, star-shaped, or fan-shaped, and much resemble the different carbohydrates, as hesperidin, inulin, etc., which separate in certain plant cells when the specimens are placed in alcohol. These crystals, however, do not separate in alcoholic material, and are to be found only in galls which have been kept in copper acetate solution. Their appearance, reactions, and a comparison with copper gallate crystals lead to the conclusion that they are identical in composition with the latter salt. (3) In the external layer or zone of specimens which are at this stage of maturity,

and have been treated with copper acetate, reddish-brown, amorphous, or somewhat crystalline masses are found adhering to the walls of the cells. These masses when amorphous are made up entirely of tannin and when somewhat crystalline contain an admixture of tannin and gallic acid.

III. When the winged insect has developed, (1) only a few layers of the cells of the central zone remain, and these contain a number of tannin vacuoles. Surrounding the latter are several (as many as 12) rows of prominent lignified cells. (2) The cells of the middle layer in specimens which are of this age and have been treated with copper acetate, contain numerous brownish-red tannin masses to which may be adhering some yellowish-brown crystals of gallic acid. But the tannin is in by far the greatest quantity in the cells of this layer and at this age of the galls. (3) The cells of the external layer also contain tannin masses.

*Conclusion.*—(a) It is well known that gallic acid occurs naturally in the nut galls (the product of *Cynips gallae tinctoriae* Olivier on *Quercus Lusitanica* Lamck.); the leaves of *Arctostaphylos Uva-ursi* (L.) Spreng., *Thea Chinensis* Sims, and of various species of *Rhus*; the fruit of *Terminalia chebula* Retzius (Myrobalans), and *Caesalpinia coriaria* Willd. (Divi-Divi); the acorn cups of *Quercus Aegilops* L. (Valonia); and may be obtained by extraction with water in the form of silky needles and asymmetric prisms. With the alkalies, alkaline earths, lead and copper salts, it forms crystalline compounds. (b) Tannic acid, on the other hand, is an amorphous substance and does not produce crystalline compounds with the salts mentioned. (c) Therefore, the crystalline compound found in the galls examined by the author is in all probability gallic acid. This appears to be formed at the expense of the starch during the chrysalis stage of the insect. With the maturing of the winged insect this is changed to tannic acid. The transformation of gallic acid into tannin appears to be one of simple condensation of two molecules of the former with the loss of one molecule of water, as follows:  $2C_6H_6O_5$  (gallic acid)  $= C_{14}H_{10}O_9$  (tannic acid)  $+ H_2O$ .—HENRY KRAEMER, *Philadelphia College of Pharmacy*.



## CURRENT LITERATURE.

### BOOK REVIEWS.

#### The Cyclopedia of American Horticulture.

THE second volume of Bailey's *Cyclopedia*<sup>1</sup> has just been issued, including the letters E-M. The general design and scope of this important work of reference has been set forth already in these pages.\* To this nothing need be added beyond the statement that the second volume is even better than the first, a natural improvement as the editors attained greater familiarity with their work and materials.

Aside from the articles treating large and horticulturally important genera like *Gladiolus*, *Iris*, *Mamillaria*, etc., there are some notable articles on general subjects, which serve to illustrate well the wise plan of the book and the thorough treatment of topics. *Forcing* is discussed in its general aspects by Professor Bailey; forcing vegetables, by C. E. Hunn; forcing fruits, by William Turner; and forcing hardy plants, by B. M. Watson. *Ferns* are treated botanically by L. M. Underwood; growing hardy ferns, by Edward Gillett and F. W. Barclay; growing tender ferns, by N. N. Bruckner. Again, in the article *Grape*, the editor writes of the general and historical aspects, while specialists in the various grape-growing regions of the north, the south, and the Pacific slope write of the practical phases of grape culture, and another of grape growing under glass. In the same way the horticultural and historical features of the *Greenhouse* are treated by the editor; Lord and Burnham write of its structural details; glass for it is discussed by J. C. Blair; and the methods of heating by L. R. Taft.

Among the biographical articles, the one on *Asa Gray*, by the editor, is a model of what such writing should be, for here Professor Bailey writes *con amore*, and with clear insight and appreciation. Morphological articles are conspicuously wanting; but perhaps these ought not to be expected. It would seem, however, that fruits might have been discussed from this point of view, as *flower* has been. *Insects* are well treated, and in part morphologically by M. V. Slingerland.—C. R. B.

<sup>1</sup>BAILEY, L. H.: *Cyclopedia of American Horticulture*, comprising suggestions for cultivation of horticultural plants, descriptions of the species of fruits, vegetables, flowers, and ornamental plants sold in the United States and Canada, together with geographical and biographical sketches. Vol. II. E-M. 4to., pp. xiv + 512-1024, figs. 744-1453, pls. 10-19. New York: The Macmillan Company. 1900. \$5.

<sup>2</sup>BOT. GAZ. 29: 282. 1900.

### Tobacco.

DER TABAK, by C. J. Koning,<sup>3</sup> treats of the commerce, manuring, culture, anatomy, diseases, and fermentation of tobacco. Well-known facts are here presented in a popular manner to those interested in the culture and trade of tobacco. Only the contents of the chapter on the so-called fermentation of tobacco are essentially new. This process the author ascribes to the action of bacteria, in accordance with the hypothesis of Suchsland. "The aroma of the tobacco is caused by facultative anaerobes in so far as we can speak of aroma in our Dutch tobacco." In this remark, from page 23, the bacteria are called "facultative anaerobes," while on page 53 we find the statement that the supposed principal generator of the aroma is an obligate aerobe. The reviewer is sorry to differ from the views of Koning, yet he has examined very carefully, with very high magnifying powers, wrapper leaves withdrawn directly from the interior of the fermenting heaps in Florida, without discovering any colonies or any coating of bacteria. The few isolated rods and cocci found on some square centimeters of leaf cannot possibly have any significance. The water content (18-25 per cent.) of fermenting wrapper leaves prevents not only the development of fungi, but still more, that of bacteria. Filler leaves are more heavily moistened, and may contain 35 per cent. of water, but even these do not show bacterial colonies when heated in closed vessels to 55° C., a temperature reached often by the heaps of fermenting tobacco leaves. Even 60° C. is often reached, and nevertheless the heaps will heat up again when taken apart and rebuilt afresh. This would be impossible if the *Bac. Tabaci* really generated the aroma, since this dies at 50° in thirty minutes, and at 60° in five minutes, according to Koning's own statement. At the same temperature also the *Diplococcus Tobaci Hollandicus*, which Koning claims improves the combustibility of tobacco, also succumbs.

In not a single instance has the author stated the water content of the tobacco when he started his bacteriological investigations. Tobacco with 50 to 60 per cent. water will no doubt readily develop bacteria, especially of the *Proteus* group which Koning found; cocci also will thrive in great numbers. Should this rotting be interrupted at the right time, a change of the odor might have occurred, which may be very desirable with Dutch tobacco. But such rotting is carefully avoided by the progressive American tobacco manufacturers, by keeping the water content so low as to avoid the action of bacteria. Under these conditions alone the oxydizing enzymes of the tobacco leaf will develop their activity, and to this the changes of odor and aroma characteristic of superior tobacco have to be ascribed.<sup>4</sup>—OSCAR LOEW.

<sup>3</sup> KONING, C. J.: *Der Tabak, Studien über seine Kultur und Biologie*. Small 4to, pp. 86. Amsterdam: J. H. & G. Van Heteren. Leipzig: Wilhelm Engelmann, 1900. M 4 (unbound).

<sup>4</sup> Compare Reports No. 59 and 65 of the U. S. Dept. of Agriculture.

## MINOR NOTICES.

THE FIRST SUPPLEMENT to Paris's *Index Bryologicus* has been issued by the house of Georg & Cie, Geneva, as one of the *Mémoires de l'Herbier Boissier*. It contains 234 pages, with innumerable entries correcting and adding to the original *Index*.—C. R. B.

THE FOURTH PART of the new edition of Weisner's *Die Rohstoffe des Pflanzenreiches*<sup>5</sup> completes the account of plant fats (42 pp.); Dr. K. Mikosch treats vegetable wax (21 pp.); Dr. A. E. von Vogl writes the tenth section on camphor (6 pp.); Dr. S. Zeisel contributes the chapter on starch (80 pp.); and Dr. Lafar's section on yeast is begun (11 pp.).—C. R. B.

THE SECOND FASCICLE of the list of the genera of seed plants, according to the system of Engler, has appeared with remarkable promptness.<sup>6</sup> In the notice of the first part in this journal<sup>7</sup> the general character of the work was stated. In the present signatures 1220 genera are listed, bringing the number up to 2490, the list beginning with *Alophia* (Iridaceæ) and ending with *Silene* (Caryophyllaceæ).—J. M. C.

RECENT NUMBERS of Engler's *Die natürlichen Pflanzenfamilien* are as follows: Number 198 contains a continuation of *Musci*, by Carl Müller and W. Ruhland, and deals as yet with the general morphological characters of the group. Number 199 contains the conclusion of the Marattiaceæ by G. Bitter, the Ophioglossaceæ by the same author, and a general discussion of fossil Filicales by H. Potonié. Number 200 and 201 (a double number) contains the completion of the Hyphomycetes, by G. Lindau, and with it the completion of the part of the first volume which deals with the Fungi.—J. M. C.

DR. J. P. LOTSY gives a résumé in English<sup>8</sup> of several articles in German and Dutch, in which he has published the results of his investigations upon the localization and formation of alkaloids in Cinchona. The chief results may be stated briefly thus. Normally, alkaloid is present exclusively as the content of living parenchyma cells, or of other cells differing but little from parenchyma. It never occurs in cells containing oxalic acid (as oxalate). Usually it is dissolved in the cell sap in young organs, but is an amorphous solid in old parts. The alkaloid is formed in the leaves and transported to the bark, where it is stored either in its original form or, after transformation

<sup>5</sup> Pp. 481-640, *figs. 89-122*. Leipzig: Wilhelm Engelmann. M 5. See BOT. GAZ. 30: 66. 1900.

<sup>6</sup> DALLA TORRE, C. G. DE, and HARMS, H.: *Genera Siphonogamarum ad systema Englerianum conscripta. Fasciculus secundus (signatura 11-20)*. Small 4to, pp. 81-160. Leipzig: Wilhelm Engelmann. 1900. M. 4.

<sup>7</sup> BOT. GAZ. 30: 67. 1900.

<sup>8</sup> Bulletin de l'Institut botanique de Buitenzorg. III. 8vo, pp. 43.

to another alkaloid. It is considered not at all impossible that the alkaloid is formed by direct synthesis, and not as a decomposition product of proteids.—C. R. B.

A SMALL PAMPHLET by W. Johannsen presents an account in popular form of a process which has already attained considerable use in forcing-houses.<sup>9</sup> The method, which extraordinarily hastens the development of shoots and flowers, consists in exposing dormant plants in a suitable chamber twice to ether vapor for 24-72 (mostly 48) hours, with 48 hours interval, the time depending on the temperature and phase of the resting period.

The first section, "Zur Orientirung über die Ruheperiod," has considerable theoretical interest. The author defines clearly the expression "resting period," and shows the erroneousness of the common idea that the riper the wood or seed of a plant is, the easier will be the budding or sprouting. He points out that the resting period has no sharp limits, but is a passage from diminished power of growth through complete rest to increased power of growth again.

In the second section detailed directions are given for the practice, which is especially applicable to syringas, azaleas, snowball, spireas, deutzia, lily of the valley, and tulips.—C. R. B.

THE FIELD COLUMBIAN MUSEUM has recently come into possession of a set of plants collected by Don José Blain on the Isle of Pines, Cuba, some time in the middle of the sixties. The list, including 185 numbers, among which are four new species (*Polygala*, *Salacia*, *Spigelia*, and *Heliotropium*), has appeared as one of the publications of the museum (1:425-439. 1900), under the title "Plantae Insulae Ananasensis," by Charles F. Millspaugh, the curator.—The same author has also issued a second paper under the title "Plantae Utowanae" (Field Columb. Mus. Bot. 2:113-135. 1900), in which he reconsiders the Cyperaceæ and Cakile of the former one (see BOT. GAZ. 29:360. 1900). The present paper takes up the two groups in the form to be used in the proposed *Yucatan Flora*, in which all of the specific descriptions are to be based upon the characters of the fruits, and accompanied by text cuts illustrating these characters. Dr. Millspaugh differs from C. B. Clarke in regarding *Mariscus* and *Torulinium* as but sections of *Cyperus*. *Cyperus* (*Mariscus*) *Caymanensis* is described as a new species. In *Cakile* ten species are recognized, two of which are new; also two new hybrids are described. The author makes the very interesting observation that the genus has laid special stress upon the development of the fruit for dissemination, and that the "evolution for floatage seems to have reached its height in the new species growing upon the Alacran shoals."—J. M. C.

<sup>9</sup> JOHANNSEN, W.: Das Aether-Verfahren beim Fröhrtreiben mit besonderer Berücksichtigung der Flidertreiberei. 8vo., pp. 28, figs. 4. Jena: Gustav Fischer. 1900. 80 pfg.

STIGMONOSE is the title of a bulletin by Albert F. Woods<sup>10</sup> in which he describes fully his studies of the disease of carnations and other pinks, formerly called bacteriosis by Arthur and Bolley, and ascribed by them to the action of *Bacterium dianthi*. The preliminary statement by Mr. Woods as to the cause of the disease and the tone of his criticisms on Arthur and Bolley's work<sup>11</sup> were criticised by this journal<sup>12</sup>. Mr. Woods has now presented the evidence on which his conclusions rest, and it entirely justifies the substance of his criticism. Moreover, the account of Arthur and Bolley's work in the bulletin is full, and the defects in it are pointed out in a way to which no exception can be taken.

Woods shows that neither fungi nor bacteria are present in the earlier stages of the disease, and though they may appear later, their presence is not constant. The punctures by aphides are responsible for the disease, as was shown repeatedly by colonizing aseptically these insects on carnations. As "bacteriosis" is misleading, stigmonose is suggested to replace it. Mr. Woods believes "that the insect injects some irritating substance of an acid or enzymic nature into the wound; that this substance causes the increase of oxidizing enzymes in the cells which it reaches, and that these enzymes interfere with the nutrition of the cell by destroying the chlorophyll and setting up other changes which finally result in death."—C. R. B.

A FRENCH TEXT on the anatomy and physiology of plants by Er. Belzung<sup>13</sup> shows that one can produce a very imposing and attractive looking book without contributing to the pedagogical advancement of a subject. The author is professor of natural sciences in the Lycée Charlemagne, and the author of various elementary text-books, two geological, one paleozoological, and one botanical, and one zoological, which have reached third and fourth editions. A corresponding work on the anatomy and physiology of animals has reached its eighth edition.

The author disclaims having written a treatise, but calls his book a work for the student in which he may obtain the fundamentals to prepare him for the study of completer works. But he will be an unfortunate student who bases his knowledge on such a book as this. Not that it is so full of errors, though it sins in this respect, but its correctness is purely mechanical. Moreover, erroneous impressions and ill-chosen points of view unfit it for its

<sup>10</sup> WOODS, A. F.: Stigmonose, a disease of carnations and other pinks. Bulletin 19, U. S. Dept. Agric., Div. of Veg. Phys. and Path. 8vo, pp. 30. *pl.* 3. Washington: Gov. Printing Office. 1900.

<sup>11</sup> A. A. A. S., Toronto meeting; see BOT. GAZ. 24: 200-205. 1897.

<sup>12</sup> BOT. GAZ. 25: 129-130. 1898.

<sup>13</sup> BELZUNG, ER.: Anatomie et physiologie végétales, à l'usage des étudiants en sciences naturelles des universités, des élèves à l'institut agronomique, des écoles d'agriculture, etc. 8vo, pp. iv + 1320, *figs.* 1699. Paris: Felix Alcan. 1900.

announced purpose. For example, the "circulation" of the sap is repeatedly described and impressed by a diagram with arrows showing the direction of the "ascending sap" and the "descending sap." The "osmotic force" is presented as "une nouvelle force," residing in the protoplasm in virtue of which it exercises "une puissante attraction." Many other similar cases might be cited from all sections.

The best thing about the book is the illustrations, most of which are excellent. But as a whole it can hardly be commended.—C. R. B.

A NEW PART (second series, Part IV) of the *Minnesota Botanical Studies* has appeared, bearing the date August 15, 1900. It contains seven papers of varying length, and is altogether a worthy member of the series. "A contribution to the knowledge of the flora of southeastern Minnesota," by W. A. Wheeler, is in the nature of a report of the work of the State Botanical Survey during the summer of 1899, and the results are presented with a well-organized ecological background, accompanied by seven excellent plates from photographs showing characteristic vegetation features. "The seed and seedling of the western larkspur (*Delphinium occidentale*)," by Francis Ramaley, is a brief morphological and histological study, illustrated by a plate. "A preliminary list of Minnesota Erysiphææ," by E. M. Freeman, catalogues nineteen species, with their hosts. K. C. Davis publishes three important revisions which have been developed in connection with the work on Professor L. H. Bailey's *Cyclopedia of American Horticulture*. They are as follows: "Native and garden Delphiniums of North America," 52 species being described, one of which is new; "Native and cultivated Ranunculi of North America and segregated genera," the genera being *Batrachium* S. F. Gray (5 spp.), *Ranunculus* L. (96 spp., two new), *Kumlienia* Greene (1 sp.), *Ficaria* Huds. (1 sp.), *Cyrtorhyncha* Nutt. (1 sp.), *Arcteranthus* Greene (1 sp.), and *Oxygraphis* Bunge (1 sp.); "A synonymic conspectus of the native and garden Thalictrums of North America," 35 species being described. The final paper is entitled "Some preliminary observations on *Dictyophora ravenelii* Burt," by C. S. Scofield, accompanied by three excellent plates, and among the conclusions reached the following is of general interest: "There is in the young mycelial threads very good evidence of the occurrence of cell fusion previous to, or in intimate connection with, the formation of the sporophore." —J. M. C.

#### NOTES FOR STUDENTS.

NAWASCHIN<sup>41</sup> has recently made a cytological study of *Plasmodiophora brassicae* Woron. *Plasmodiophora* is a parasitic myxomycete which causes various deformities and distortions in the roots of its host. *P. brassicae* is

<sup>41</sup>Beobachtungen über den feineren Bau und Umwandlung von *Plasmodiophora Brassicae* Woron. im Laufe ihres intracellularen Lebens. *Fora* 86: 404-427. *pl.* 20. 1899.

the cause of "club root" in cabbages and turnips. Woronin, who studied this form more than twenty years ago, found that it does not form sporangia, but that the spore masses lie free in the cells of the host. Eycleshymer, in *Jour. Mycol.* 7:79-88. 1892, gives a clear account of its life history and distribution in the United States. The present paper is concerned with the more minute details. Infected roots were cut into very small pieces and were treated with Flemming's stronger solution for twenty-four hours. The author thinks that the advantages of a more prolonged treatment are entirely imaginary. Sections were cut 2-3  $\mu$  in thickness, and were fastened to the slide with distilled water without any further fixative. The Flemming triple stain gave the best results. Dilute Delafield's haematoxylin, followed by eosin in clove oil, and also the gentian-violet method according to Gram, gave good results. The peculiar method of nuclear division in the vegetative amoebae is worthy of special mention. In the resting condition this nucleus has a membrane, a nucleolus, and an extremely delicate chromatin network. As division begins, clearly differentiated chromatin granules appear in the place of the network, the granules having no genetic connection with the nucleolus. A plate evidently derived from the chromatin granules is then seen near the nucleolus. At this stage there is a sort of one-sided "achromatic figure" with its base resting upon the chromatin plate and its apex at the nuclear membrane, but the figure afterward acquires a symmetrical aspect, the drawings in the plate bearing considerable resemblance to bipolar spindles. The nucleolus then divides transversely, and the two resulting bodies take positions on opposite sides of the chromatin plate, which is now a homogeneous disk with chromatin granules imbedded in its periphery. The chromatin plate then splits, and the two parts, each accompanied by a nucleolus, move toward the poles of the spindle, and two daughter nuclei are formed. This method of division is of greater interest because the nuclear division in the plasmodium is simultaneous and of the usual bipolar mitotic type. The author's summary of the entire paper is about as follows:

1. The group of infected cells arises by the repeated division of a primary infected cell.
2. During the growth of the infected cells numerous multinucleate amoebae appear, which multiply without fusing to form a plasmodium.
3. In this condition the amoebae of *Plasmodiophora* are remarkable for their structure, and especially for their unique mode of nuclear division.
4. The mode of nutrition of the amoebae seems to be different from that in other *Myxomycetes*.
5. As the amoebae fuse into a plasmodium, characteristic changes take place in the structure of the body and in the nuclei.
6. The formation of a plasmodium takes place only after the host cell is exhausted.

7. In the mature plasmodium spore formation is preceded by repeated nuclear division of the typical mitotic sort.

8. In the first period of its development the parasite does not kill the host cell, but merely causes it to hypertrophy.—CHARLES J. CHAMBERLAIN.

HERBERT J. WEBBER, who has long been investigating the subject of plant-breeding for the Division of Vegetable Physiology and Pathology, U. S. Department of Agriculture, has published<sup>15</sup> his results upon xenia in maize, which means the immediate effect of pollen upon structures outside of the embryo. His experiments are by no means complete, but they have already yielded suggestive results, and the author is justified in their publication by the fact that the subject has been brought under discussion by the discovery of double fertilization, and its application by de Vries and Correns to the explanation of xenia. Mr. Webber had already reached the same conclusion independently, and was collecting a large amount of experimental data to substantiate his more leisurely developing paper.

It seems that the name "xenia" was applied to this phenomenon by Focke.<sup>16</sup> While it is claimed to be a somewhat common phenomenon in many plants, there are very few cases on record that are not open to doubt, and in no plant is its occurrence so well substantiated as in maize. The experiments of Mr. Webber have been conducted since 1898, in Washington and at the Nebraska Agricultural Experiment Station. The greatest care was used to obtain pure races and to prevent the access of foreign pollen. Full details are given of about thirty experiments which yielded pertinent results, and the paper closes with their discussion.

The author abundantly confirmed Körnicke's statement that xenia is shown only in the endosperm. Color in the endosperm is frequently transmitted by the pollinating parent, but color in the pericarp is not. The chemical composition of the endosperm is also greatly affected by the pollinating parent, sweet corn crossed with dent or flint races producing smooth grains with starchy endosperm, and *vice versa*. All of the experiments favor the theory that xenia in maize is caused by the fertilization of the endosperm nucleus by one of the male cells. All of the grains showing xenia proved to be true hybrids. In the case of spotted grains the author proposes an interesting hypothesis by way of explanation. He suggests that the male nucleus may fail to fuse with the polar nuclei, and in such a case may be able to form a spindle and divide separately. In this event two races of free nuclei would be formed, and when the parietal placing and tissue formation begin, the two races might become intermixed. A second hypothesis explaining this phenomenon suggests that the male cell fuses with but one of the polar nuclei,

<sup>15</sup>Xenia, or the immediate effect of pollen, in maize. Bulletin 22, with four plates (one colored). Issued September 12, 1900.

<sup>16</sup>Die Pflanzen-Mischlinge 511. 1881.



the other polar nucleus dividing independently. This also would result in two races of nuclei which might become more or less intermixed before fixation in a tissue.

We await with expectation the minute investigation of the structures concerned, which should settle the question of double fertilization for maize.—J. M. C.

THE POWER of the infusoria to adjust themselves to certain changes in their nutrient medium is the subject of a paper by Atsushi Yasuda.<sup>17</sup> From a nutrient fluid in which they were growing normally, the infusoria were transferred to solutions of the following substances: saccharose, glucose, lactose, glycerin,  $\text{KNO}_3$ ,  $\text{NaNO}_3$ ,  $\text{MgSO}_4$ ,  $\text{KCl}$ ,  $\text{NaCl}$ , and  $\text{NH}_4\text{Cl}$ , in varying concentration. The organisms used for the experiments were *Euglena viridis*, *Chilomonas paramœcium*, *Mallomonas Plasslii*, *Colpidium colpoda*, and *Paramœcium candatum*. In general, excepting *Euglena*, these can withstand about 6 per cent. of glucose, while *Euglena* withstands 11 per cent. With the other sugars the ratio is nearly the same, but with glycerin it is about  $\frac{4}{11}$  instead of  $\frac{6}{11}$ , as above. In the case of the electrolytes it is still greater, usually however less than unity. *Aspergillus niger* withstands over 50 per cent. of glucose and 21 per cent. of  $\text{NaNO}_3$  (Eschenhagen), and *Zygnema* adapts itself to 50 per cent. of saccharose and 20 per cent. of glycerin (Klebs). Thus we see that in this respect *Euglena* stands intermediate between the other organisms here studied and the lower algæ and fungi, studied by Eschenhagen, Klebs, and Richter. This may be of interest to those attempting to decide the question of the animal or plant nature of *Euglena*.

If we use De Vries' coefficients for comparing osmotic pressures, the solutions at the limit of adaptability are, in a very general way, nearly isosmotic. The fact that in the author's results there are many very pronounced exceptions to this statement is not to be wondered at, since he has not only subjected the organism to a strong solution of the substance to be tested, but has also deprived it of its proper food supply. This, it would appear, is a very weak point in all such experimentation as the present. All these results were obtained when the infusoria must have been in an unhealthy condition. In general, the limit to the adjusting power seems to be of an osmotic nature. When the organism is first placed in one of these strong solutions its membrane becomes wrinkled, owing, doubtless, to extraction of water. Later these folds disappear; this is apparently the effect of an osmotic change in the protoplasm, adapting itself to the new conditions. The more concentrated the solution becomes, the more rounded is the form of the organism, and its outline becomes uneven.—BURTON EDWARD LIVINGSTON.

<sup>17</sup> Studien über die Anpassungsfähigkeit einiger Infusorien an concentrirte Lösungen. Jour. Coll. Sci. Imp. Univ. Tōkyō 13: 101-140. pls. 10-12. 1900.

DR. TH. BOKORNY discusses<sup>18</sup> the various modes of storage of proteids and their microchemical relations. Proteids soluble in 5-10 per cent. salt solution (globulin) are stored in the proteid grains and crystals ("aleurone" and "crystalloids") of seeds. Proteids insoluble in NaCl solution have not been observed in proteid grains. Neither "active proteid" nor fat could be detected in the proteid grain itself; the fat is associated with the general proteids of the seed, probably with the plasmatic proteids. The plant caseins seem not to occur in the proteid grains, for these dissolve completely in NaCl solution, whereas the caseins are not soluble therein. The glutens of the cereals constitute a special case; they dissolve in 70-80 per cent. alcohol, a fluid which usually serves to precipitate proteids. Peptone was not recognizable in resting seeds. It and peptonizing enzymes occur in plants only exceptionally, as in fungi and carnivorous plants. Simple amides (asparagin, tyrosin, leucin, etc.) are well known in seeds and are widely distributed in vegetative parts. They appear to be the first decomposition product as well as the first formative stage of the proteids.—C. R. B.

L. KNY has been unable to find any traces of the intercellular living protoplasm said by Baranetzki to be observable in the intercellular spaces of young stems of *Myriophyllum spicatum* and *Ceratophyllum demersum*, and in young petioles of *Nuphar luteum*. Sauvageau also claimed to have found intercellular protoplasm in roots of *Naias major*. Kny examined a number of water plants and says: "In no case was I successful in observing living protoplasm (whether with or without nuclei or chromatophores) as a lining in the young or full grown air spaces except when its origin from the surrounding cells was in the highest degree probable. Even in the most advantageous covered preparations, in which the protoplasm within the cells adjoining the air spaces proved motile for several (in extreme cases fourteen) days, no sign of self-movement in the periphery of the air spaces was to be seen. The existence of a living extracellular plasma in the large air spaces of water plants must remain improbable until proof more convincing than at present is forthcoming."—C. R. B.

HERMANN FISCHER concludes his paper on "Der Pericykel in den freien Stengelorganen"<sup>19</sup> as follows:

1. In about 32 per cent. of the dicotyledons investigated a more or less perfect endodermis may be recognized marking the distinction between cortex and central cylinder. The so-called pericycle, by its position between the limit of the cortex and the ring of vascular bundles is allied with the pericambium of the root. Considered histologically, genetically, and as a formative region, no characters common to pericycle and pericambium can be predicated.

<sup>18</sup> Bot. Cent. 82: 289-306. 1900.

<sup>19</sup> Jahrb. f. wiss. Bot. 35: 1-27. 1900.

2. In monocotyledons, conifers, and 68 per cent. of the observed dicotyledons no characteristic limit of the cortex is recognizable. The ring of mechanical tissue in the monocotyledons is from no point of view allied to the pericambium.—C. R. B.

DR. F. CZAPEK describes<sup>20</sup> a thermostat for use in experiments involving the use of a clinostat. The apparatus consists of a rectangular iron sand bath on four legs with leveling screws. This is heated by a microburner and over it is set a copper box sheathed with asbestos and equipped with the usual thermoregulator and thermometer. The ends of the box are slit to pass over the axis of the clinostat which carries the experimental plants. These slits can be closed by slides, except a circular aperture, which can be centered about the axis of the clinostat by means of the leveling screws of the base. Temperatures may be maintained constant within a degree for several days. The apparatus may easily be modified to furnish one-sided or uniform illumination by making part or all of the box of glass. A saturated atmosphere can be obtained by using water instead of sand in the pan.—C. R. B.

CHARLES PALMER NOTT has published an account<sup>21</sup> of the species of *Nitophyllum* of California. The paper is more than a description of the species, for it contains a general discussion of the generic characters and of the geographical distribution. It seems that this genus of the red algæ has a general distribution throughout the oceans of the globe. About seventy species are known, ten of which occur on the west coast of North America, and eight of these are limited to California or neighboring shores. *N. corallinarum* is described as a new species. The plates are photolithographs, and show the plant forms and even the texture excellently.—J. M. C.

<sup>20</sup> Ber. d. deutsch. bot. Gesells. 18:131. 1900.

<sup>21</sup> Proc. Calif. Acad. Sci. III. 2:1-46. pls. 1-9. 1900.

## NEWS.

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DR. B. M. DUGGAR of Cornell University has been elected a member of the German Botanical Society.

DR. H. AMBRONN, private docent in the University of Leipzig, has been called to the assistant professorship of botany in the University of Jena.

THE DIVISION of Vegetable Physiology and Pathology has secured a table at the Marine Biological Laboratory at Woods Hole for the use of its staff during the summer months.

BOTANY, a text-book for schools by Professor L. H. Bailey, is announced by the Macmillan Company. It is stated that "this book has all the popular features of the old time text-book—organography, morphology, elementary anatomy, and a flora—but it presents them in a new way."

DR. HERMANN VON SCHRENK is continuing his work for the Division of Vegetable Physiology and Pathology on the diseases of forest trees. As a result of the work of last year two bulletins have been prepared and are now going through the press. Dr. von Schrenk is at present on the Pacific coast, where he will remain for several months collecting information and arranging for more extensive work later.

MR. M. A. CARLETON, of the Division of Vegetable Physiology and Pathology, has completed the installation of the cereal exhibit of the Department of Agriculture at the Paris Exposition, and is now in southern Russia collecting grains, which will be distributed by the Section of Seed and Plant Introduction. A number of the varieties of cereals obtained by Mr. Carleton during his former trip to Russia have proved of value.

EARTH AND AIR is the title of a new monthly journal devoted to meteorology. The general purpose of the journal, as indicated by the prospectus and by the contents of the first number, is so pertinent to the work of ecologists and students of plant diseases, that the attention of botanists should be called to it. In the first number, Mr. Albert F. Woods, of the Division of Vegetable Physiology and Pathology, U. S. Department of Agriculture, writes concerning "Weather and plant diseases." The yearly subscription price is one dollar, and the office of publication is Penn Yan, N. Y.

## BOTANICAL GAZETTE

NOVEMBER, 1900

ON THE NATURE OF THE STIMULUS WHICH CAUSES  
THE CHANGE OF FORM IN POLYMORPHIC GREEN  
ALGÆ.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY  
XXII.

(WITH PLATES XVII AND XVIII)

BURTON EDWARD LIVINGSTON.

## Introductory.

ALTHOUGH the work of Klebs and others<sup>1</sup> has demonstrated clearly that there exist a number of polymorphic species among the green algæ, yet up to the present time the exact nature of the stimulus which brings about the change in the manner of growth has not been determined. To throw some light upon this question the experiments to be described in the present paper were begun. They were carried on in part at the Botanical Laboratory of the University of Michigan, during the spring and summer of 1898, and in greater part at the Hull Botanical Laboratory of the University of Chicago, during the year just past.

<sup>1</sup>FAMINTZIN, A.: Die anorganische Salze als Hilfsmittel zum Studium der Entwicklung niederer chlorophyllhaltigen Organismen. *Mélanges biol. St. Petersbourg* 8:225. 1871.

CIENKOWSKY, L.: 1. Zur Morphologie der Ulothricheen. *Mélanges biol. St. Petersbourg* 9:531. 1876.

———: 2. Ueber Palmellenzustand bei *Stigeoclonium*. *Bot. Zeit.* 34:17. 1876.

GAY, F.: Recherches sur le développement et la classification de quelques algues vertes. Paris, 1891.

KLEBS, G.: Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen. Jena, 1896.

### Descriptive.

A species of *Stigeoclonium* (perhaps a form of *S. tenue*), found growing with *Pleurococcus*, etc., on moist bark at Ann Arbor, was chosen for experimentation. It shows two very distinct and easily distinguishable forms according to the culture medium in which it is grown. The normal form is that of *Palmella*. A culture of this form shows the surface of the fluid covered with spherical cells 12 to 15  $\mu$  in diameter. The cells multiply by division in planes generally vertical and at right angles to each other, and the daughter cells separate more or less completely after division is accomplished (*figs. 1, 6, 12, 27*). The walls are quite thick and somewhat gelatinous on the exterior, though not by any means so markedly as in the form studied by Cienkowski (*l. c.*, 2), and the protoplasm is quite coarsely granular. The chloroplast has the form of a hollow spherical shell with an opening in one side, which has a diameter about equal to the sphere's radius, varying somewhat in different cells. Sometimes the opening is nearly a great circle of the sphere (*fig. 1, a, b*). This opening in the chloroplast is probably the "bright spot" described by Cienkowski. There is always a pyrenoid present in each cell, and sometimes two are found. This body has a diameter of about 3  $\mu$ , and lies in a thickened region in the chloroplast. When the cell is about to divide the chloroplast separates into two parts, each part taking a half of the original pyrenoid, and a wall forms between the two portions (*fig. 1, c*). The plane of division always intersects the plane of the opening in the chloroplast. Then the two hemispherical cells become more and more spherical, splitting the wall between them along its middle lamella, until finally, if nothing prevents, they come to lie as two separate spheres side by side (*fig. 1, a*). Very often division proceeds more rapidly than this rounding off, and there results a group of four or more cells making contact with each other in plane surfaces (*fig. 6*). Often this process is continued without any parting till there results a plate of cells spread out over the surface of the nutrient medium. Sometimes, too, the planes of division lie parallel to the surface of the

medium as well as perpendicular to it, and a parenchyma-like mass of polygonal cells is formed, projecting down into the fluid and up into the air (*figs. 6, 24*). After division the chloroplast enlarges until it occupies about as much of the daughter cell as the mother chloroplast did of the mother cell. The form just described will be termed throughout this paper the *palmella form*.

In the other form we have a very different mode of growth. The cells are not spherical, but cylindrical; they divide, excepting at the origin of a branch, only by transverse planes, and show no tendency to break apart, but remain closely connected to form branching filaments (*figs. 2, 8, 18*). This will be termed in the following discussion the *filamentous form*. The cells of the filaments are  $4.5$  to  $10\mu$  in diameter and from two to four times as long. The longest filaments are  $175$  to  $200\mu$  long and their branches extend in all directions. No true hairs have been seen, but often the tips of the filaments are very narrow. The protoplasm is not granular, as in the *palmella form*, but usually contains two to seven refractive bodies smaller than the pyrenoid, but looking much like it. They are probably of an oily nature. They have a diameter of about  $15\mu$ , that of the pyrenoid being, as in the other form, about  $3\mu$ . The chloroplast is elongated to suit the cell and becomes somewhat trough-shaped; lying along one side of the cylindrical wall it reaches about one half way around the cell and usually partially covers one end, sometimes both. Its opening is thus longer than in the other form. The pyrenoid lies somewhere in a thickened part of the chloroplast.

Reproduction in both forms is accomplished by comparatively large asexual biciliate zoospores. These are the microzoospores of Klebs. I have observed no conjugating zoospores. The chloroplast of the mother cell divides into four to eight parts along curiously parallel planes running nearly at right angles to the plane of the opening (*fig. 1*). Each of these portions, with its share of the protoplasm, becomes a zoospore. After division is complete and the zoospores are nearly formed, the daughter

chloroplasts often lose much of their bright green color and become pale. Palmelloid cells usually produce seven or eight zoospores; the number produced by a filament cell depends upon its size. The zoospores are ellipsoidal to spherical when discharged into the water by the rupture of the parent wall, and immediately begin to move about, swimming by means of two long cilia (*fig. 3*). Each contains a pyrenoid, and often several smaller granules; the chloroplast can be seen lying against the wall opposite the origin of the cilia and extending in its usual cup-shaped form somewhat more than half way around the cell. The dimensions of motile zoospores are from  $3 \times 6 \mu$  to  $6 \times 9 \mu$ . After swimming freely for several hours, the motion of the cilia becomes sluggish and finally ceases altogether; the zoospore comes to rest and assumes the spherical form. The diameter is now not far from  $6 \mu$ . The chloroplast then regains its original bright color, the cell enlarges, and, if conditions are favorable, may go over imperceptibly into the palmella form. The cell thus produced may divide a number of times, as described above, and then produce more zoospores; or it may produce them immediately upon the attainment of its full size, or even earlier; or it may become a resting cell and remain quiescent indefinitely.<sup>2</sup> However, a zoospore does not usually show this mode of growth. It generally elongates as it lies on the surface (or sometimes at the bottom) of the fluid, and becomes a cylinder with rounded ends. As it grows longer it bends so that it becomes somewhat crescent-shaped, semicircular, or even horse-shoe shaped, and soon divides into two cells by a transverse wall near its middle (*figs. 8, 32*). Thus a filament is started, which soon branches. Its cells produce zoospores sooner or later, according to conditions.

### Methods.

Pure cultures were first obtained by growing in nutrient solution in suspended drops; then the material was transferred to

<sup>2</sup> KLEBS, G.: Loc. cit.

PRINGSHEIM, N.: Ueber die Dauerschwärmer des Wassernetzes. Monatssch. d. Berliner Akad. 1860.



small, loosely covered glass dishes containing from 5<sup>cc</sup> to 10<sup>cc</sup> of solution. The cultures stood on glass shelves close against the panes of an east or west window, and were always shaded from the direct rays of the sun by a curtain of white muslin, which was left constantly in position during the high temperatures of the summer months. Examination of cultures was made from time to time by transferring the dish with its contents directly to the stage of the microscope and using an objective of medium low power.

The culture media used were modifications of the well-known fluid of Knop,<sup>3</sup> consisting of the following salts: calcium nitrate, four parts; magnesium sulfate, one part; potassium nitrate, one part; di-potassium acid phosphate, one part; iron, a trace. Owing to the extreme weakness of the solutions to be used, it was deemed advisable to secure at the outset, as nearly as might be, the exact proportions of the constituent salts here given, and to this end the following method was adopted. By reference to tables of the physical properties of solutions,<sup>4</sup> the corresponding specific gravity and gram-molecular strength of solutions of the first three of the above salts were found. By the use of these data a stock solution of each of these compounds was made up, so that it contained a specific number of gram-molecules per liter. The specific gravity bottle was used in these determinations, and temperature was always taken into account. For the fourth salt in the list data could not be found; therefore it was formed in solution from normal solutions of phosphoric acid and potassium hydroxid. The physical constants for these could be found. The accuracy of this method for producing  $K_2HPO_4$  was tested by volumetric analysis and found to be satisfactory. The specific gravity of a normal solution of this salt so made was found to be 1.13207 at 15° C. Redistilled water was used throughout all of the work.

The stock solutions thus prepared were kept in flasks tightly stoppered with rubber; their specific gravity was taken from

<sup>3</sup> KLEBS, G.: op. cit., p. 8.

<sup>4</sup> E. g., those given by WHETHAM, W.C.D., *Solution and Electrolysis*, 215. Cambridge. 1895.

time to time, and water was added as it was lost by evaporation. Knowing the gram-molecular, and hence the percentage, strength of these stock solutions, any strength of Knop's solution may readily be made up from them; and solutions so made up are much more accurate in their proportions than those made in the ordinary way; for in dealing with salts such as magnesium sulfate and calcium nitrate the amount of water contained in the compound as taken from the laboratory bottle is always an uncertain quantity. All troubles arising from crystalline and amorphous, as well as deliquescent, salts may thus be readily avoided. The exact amount of ferric salts in my solution was not determined. The transfers of material from an old culture to a new were made, after a pure culture was obtained, by means of a needle. This was first heated in a flame and then cooled by plunging in the new culture fluid. Bits of steel always scaled off in this operation, and these furnished sufficient iron for the plants.

In making up culture media from the stock solutions of the constituent salts, it is necessary to dilute as far as possible before bringing the  $K_2HPO_4$  and  $Ca(NO_3)_2$  together. As was remarked by Klebs, this avoids, in a great measure, the separating out of quantities of  $CaHPO_4$ . This method is not completely satisfactory, however, and it would be better so to modify the proportions as to avoid the white precipitate entirely. I have planned to do this in future work. A solution made up of the quantities of the salts given by Knop dissolved in 93 parts of water is, of course, a 7 per cent. solution (since the iron is of such small amount it may be disregarded). This was further diluted to 1 per cent., 1.5 per cent., and 2 per cent. for growing the stock material.

#### Investigation.

At the outset it was found that if material in the palmella form were transferred from a 1 per cent., 1.5 per cent., or 2 per cent. solution where it had been growing for some time, to a solution of less than 0.5 per cent., the plants responded to the

change in solution strength, and sent out long filaments from the original palmella masses (*figs. 7, 15, 16*). Also the palmella cells produced numerous zoospores. These germinated, as previously described, to produce, not the parent form, but the widely different filamentous one. Conversely, it was found that if filaments, produced as above, and growing healthily in a 0.2 per cent. or 0.25 per cent. nutrient solution, were changed to one of 1 per cent., 1.5 per cent., or 2 per cent. strength, the response was scarcely less well marked than in the other case; the cells of the filaments soon became spherical, and changed to the palmella form by dividing in both longitudinal and transverse planes (*figs. 4, 5*). In the strong solutions few or no zoospores were produced. Where a few were produced they changed, either directly or after the first or second division, to the palmella form.

With these two facts in view the attempt was made to determine where, in this change of solution strength, lies the necessary stimulus for the production of the corresponding change of form. A stimulus arising from a change of solution strength (always retaining the same salts in the same proportion) may be of any one of three different natures. (1) It may be of a chemical nature: *i. e.*, it may be due to a change in the amount of salts with which the organism is supplied. This implies three possibilities: (*a*) the plant may be affected by the increase or decrease in all the inorganic salts. (*b*) Again when such change is made it may be the change in amount of a single salt, say potassium nitrate, to which the plant responds. There are four possibilities under this head, one for each of the four salts used. (*c*) Again, it may be possible that neither of the preceding suppositions is true, but that the response is due, not to all the changes together, nor to any one alone, but to a combination of two or three of them. For example, it may be a change in the amount of potassium phosphate together with that of calcium nitrate, which acts as a stimulus. We know that, excepting in very weak solutions, these two salts react chemically upon each other, so that it is at least conceivable that an

increase or decrease in both of them together might influence the plant differently from a corresponding change in only one. Under this head there are no less than ten possibilities, six taking the salts by twos, and four by threes. (2) Further, the stimulus may be of a physical nature and due, not to the change of salts at all, but to a change of osmotic pressure upon the living cell. This osmotic change may be effective in several ways. (a) It may be that the response is due to a change in the osmotic pressure of the solution in *general*. When the saline constituents of the solution are increased or decreased as a whole there is a corresponding change in the so-called osmotic strength. (b) However, it may be, as suggested by Copeland's recent work,<sup>5</sup> that the plant is more influenced by osmotic pressure when this is derived from one salt than by the same pressure derived from another. And if this be the case, then this influence is as complicated a one as that of the change in the conditions enumerated under (1) above. (3) Finally, the response in the plant may be due both to the stimuli from chemical composition and to those from osmotic pressure, a combination of (1) and (2) above.

My experiments were devised to determine primarily whether the stimulus is a chemical or a physical one. For this it is necessary to have solutions in which the relative and absolute amounts of saline constituents can be varied without changing the osmotic pressure of the salt content as a whole, and this must be brought about, as far as may be, without the introduction of any new conditions. It is necessary first to know the osmotic pressure of the complete Knop's solution. Now the pressure of any weak solution of several constituents is equal, at least approximately, to the sum of the partial pressures of the constituent salts, as these pressures would exist if the salts were separately put into a simple solution whose volume equaled that of the complex one. This principle makes it a simple matter to calculate the pressure of complex solutions of

<sup>5</sup>COPELAND, E. B.: The relation of nutrient salts to turgor. BOT. GAZ. 24: 399. 1897.

non-electrolytes. But the pressure of a simple solution of an electrolyte is not so easily calculated. On account of dissociation, such pressure is much greater than that of a solution of a non-electrolyte of the same gram-molecular concentration. It was this fact with which DeVries<sup>6</sup> was dealing when he derived his so-called isotonic coefficients. But since his range of concentration was very limited, he was unable to get at the truth of the matter; and the result is, that, though his coefficients may be, and doubtless are, true for a certain strength of solution, yet, since dissociation itself varies with the concentration, they are not true in general. However, in weak solutions dissociation becomes nearly complete, and if we assume that it is complete we shall probably be able to approach the truth much more nearly than by following the method of DeVries. My solutions are all very weak, and I have no doubt that my approximations by this method are, as a rule, quite nearly true. By assuming complete dissociation, the calculated osmotic pressure for any solution becomes that of an equimolecular solution of a non-electrolyte, multiplied by the number of ions derived from a molecule of the salt under consideration. This makes the calculation very simple. Taking the pressure of one gram-molecule per liter of some undissociated substance as a unit,  $N$ , table I gives the partial pressures of the complete solution which was used as standard. This is practically the same as a so-called 2 per cent. Knop's solution as usually made up. In the pressure columns,  $a$  is derived by assuming ionization complete;  $b$  by DeVries' method; and  $c$  gives the actual pressures for those salts for which data of dissociation could be found. The discrepancy between  $a$  and  $b$  lies almost wholly in the  $\text{Ca}(\text{NO}_3)_2$ . Here we should make a greater error in using DeVries' coefficients than in assuming complete dissociation. Column  $n$  gives the number of ions derived from a molecule,  $i$  is the ratio between the actual osmotic pressure and that of an equimolecular solution of a

<sup>6</sup>DEVRIES, HUGO: *Jahrbücher für wiss. Bot.* 14: 427. 1884.

DONDERS and HAMBURGER: *Zeit. f. Physikal. Chemie* 6: 319. 1890.

non-electrolyte.  $N$  at  $0^{\circ}\text{C.}$ , is  $1686^{\text{cm}}$  of mercury, or 22.18 atmospheres.

TABLE I.

Salt	Gram-molecule per liter	$n$	Partial pressures in terms of $N$			
			$a$	$b$	$c$	$i$
$\text{Ca}(\text{NO}_3)_2$ - -	.07143	3	.21429	.28571	.175004	2.45
$\text{KNO}_3$ - - -	.02857	2	.05714	.08571	.055712	1.9
$\text{MgSO}_4$ - - -	.02381	2	.04762	.04762	.035712	1.5
$\text{K}_2\text{HPO}_4$ - -	.01664	4	.06655	.06736	.....	....
Total pressure - - -	-	-	.38559	.48641		

This 2 per cent. strength is the most concentrated solution I have used. Anything stronger kills the alga almost immediately. As we decrease the concentration below this standard 2 per cent. strength the dissociation becomes more and more complete. Thus as the concentration approaches zero as a limit the value of  $i$  approaches that of  $n$ . The value of  $i$  for the different concentrations used, as far as they can be calculated from the physical data at hand, are given in table II. Values for  $\text{H}_3\text{HPO}_4$  are inserted for comparison, in place of those for  $\text{K}_2\text{HPO}_4$ . Table II also shows the total pressures of the various percentages in terms of  $N$ . The pressures in column  $a$  will be used throughout this paper, but instead of  $0.3856 N$  I shall write  $3856 N \times 10^{-4}$ , etc.

TABLE II.

Total pressures in terms of $N$ .		"Per cent."	Values of $i$ calculated from conductivity data				
$a^7$	$b$		$\text{Ca}(\text{NO}_3)_2$	$\text{KNO}_3$	$\text{MgSO}_4$	$\text{K}_2\text{HPO}_4$	$\text{H}_3\text{PO}_4$
.38559	.486405	2	2.45	1.9	1.50	....	2.04
.289193	.364804	1.5	2.5 (?)	....	1.56	....	....
.192795	.243203	1	2.55	....	....	....	3.02
.096398	.121601	.5	2.66	1.92	....	....	....
.048199	.060801	.25	2.75(?)	....	1.68	....	....
.019279	.024320	.1	2.9	....	1.83	....	....
.00964	.013160	.05	3	2	1.89	....	4

<sup>7</sup> The meaning of  $a$  and  $b$  is the same as in table I.

Having shown thus the partial and total pressures of the normal solution, I shall now describe the modifications of this solution which I have used. Four solutions were made up according to the formula given above, excepting that each was deficient in one of the four constituent salts. All of one salt, it was thought, should not be omitted, since then, as in higher plants, pathological responses would probably ensue. In these solutions the deficient salt was reduced to one tenth its normal quantity. The decrease in osmotic pressure, caused in each instance by this diminution of one salt, was calculated by the two methods just spoken of, and of each of the three other salts a sufficiency was added to increase the pressure by an amount equal to one third of the calculated decrease. Two full sets of these solutions were made up and used, one by assuming dissociation complete, the other by De Vries' method. These two solutions were identical in their effects upon the plant. Thus finally we have four solutions in each of which a deficiency of one salt is obtained, and the osmotic pressure of the solution is kept up to normal by an increase in the amounts of the other three salts. These amounts are not equally increased by weight, but according to the pressure which they produce when in solution. I shall designate these solutions as follows: A, deficient in  $\text{Ca}(\text{NO}_3)_2$ ; B, deficient in  $\text{KNO}_3$ ; C, deficient in  $\text{MgSO}_4$ ; D, deficient in  $\text{K}_2\text{HPO}_4$ . K will denote the normal solution as given by Knop. The pressure will be expressed in terms of  $N$ .

The greater part of my work was done in a comparison of the effects upon the plants of the four solutions just described with the normal one of Knop. They were all diluted, for use in the cultures, to the various strengths given in table II. Also a number of tests were made with solutions of a single salt, using the four salts with which we have been dealing. These simple solutions were made up so as to have the same osmotic pressure as the complex ones just described. They will be designated as follows: E, a solution of  $\text{Ca}(\text{NO}_3)_2$ ; F, of  $\text{KNO}_3$ ; G, of  $\text{MgSO}_4$ ; H, of  $\text{K}_2\text{HPO}_4$ . Calculations for these were made only by De Vries' coefficients.

In all, 278 cultures were made and record kept of each for five to fifty days. They were made at all seasons of the year and in two different years separated by a period of nine months, during which the material was kept alive and changes made from time to time but no records kept. It will be seen that there are two possibly very different questions to be answered in the problem before us. First, what are the conditions that bring about a change from the palmella form to the filamentous form; second, what are the conditions that bring about the reverse change? I shall divide the experimental data which are to follow into two groups corresponding to these two questions. Details of typical responses are given in the explanation of plates.

#### A. RESPONSES OF THE PALMELLA FORM.

In table III are given the results of fifty-five transfers of palmelloid cells into the different solutions. For convenience, all cultures having the same osmotic pressure are brought together. In the left hand column the letters denote the chemical content of the fluid, as just described. The number of cultures made is given in the second column. In the third is recorded the number of cultures in which the cells multiplied but remained of the palmella form after twenty to twenty-five days. Columns four and five (*Filaments*) give the number in which filamentous branches were produced from the original masses in five to twenty-five days. Those in column four produced few filaments, but continued mostly as palmella; those in column five produced many filaments. In columns six and seven (*Zoospores*) are the numbers which produced few or many zoospores within fifteen to twenty days. Column eight contains those which produced no zoospores for twenty-five days or more. The last two columns record cultures on which definite observations are lacking, the first with regard to the production of zoospores, the second with regard to the production of filaments at the edges.



TABLE III.

Pressure $96 N \times 10^{-4}$ or 16.19 cm. Hg.										Pressure $1928 N \times 10^{-4}$ or 323.71 cm. Hg.									
Solution	Cultures	Palmeila	Filam'ts		Zoospores				Filaments ?	Solution	Cultures	Palmeila	Filam'ts		Zoospores				Filaments ?
			Few	Many	Many	Few	None	Doubtful					Few	Many	Many	Few	None	Doubtful	
K	12	1	2	4	10	2	..	..	5	K	5	3	..	2	2	1	2	..	..
A	5	..	1	4	5	..	..	..	..	A	4	1	3	..	..	..	4	..	..
B	5	..	2	3	3	2	..	..	..	B	4	2	..	2	..	..	4	..	..
C	5	..	..	5	5	..	..	..	..	C	4	..	2	2	..	2	2	..	..
D	5	2	..	3	5	..	..	..	..	D	4	2	2	..	1	..	3	..	..
E	8	..	2	3	7	1	..	..	3										
F	2	..	2	..	2	..	..	..	..										
G	2	..	1	1	2	..	..	..	..										
H	2	..	1	1	2	..	..	..	..										
Pressure $193 N \times 10^{-4}$ or 32.37 cm. Hg.										Pressure $2892 N \times 10^{-4}$ or 487.59 cm. Hg.									
K	2	..	1	1	..	1	..	1	..	K	5	2	3	..	..	..	5	..	..
A	2	..	1	1	..	1	1	..	..	A	5	..	3	2	..	..	5	..	..
B	2	..	2	..	..	2	..	..	..	B	5	1	4	..	..	..	5	..	..
C	2	..	2	..	..	..	2	..	..	C	5	..	5	..	..	1	4	..	..
D	2	2	..	..	..	2	..	..	..	D	5	3	2	..	..	..	5	..	..
Pressure $482 N \times 10^{-4}$ or 81.27 cm. Hg.										Pressure $3856 N \times 10^{-4}$ or 647.42 cm. Hg.									
K	2	..	..	..	2	..	..	..	2	K	2	2	..	..	..	..	2	..	..
Pressure $964 N \times 10^{-4}$ or 161.86 cm. Hg.										A	2	1	..	1	..	..	2	..	..
K	7	2	1	3	5	1	..	1	1	B	2	..	2	..	..	..	2	..	..
A	5	..	3	2	2	1	2	..	..	C	2	..	2	..	..	..	2	..	..
B	5	2	..	3	5	..	..	..	..	D	2	2	..	..	..	..	2	..	..
C	5	1	1	3	2	1	2	..	..										
D	5	3	1	1	3	..	2	..	..										
E	2	2	..	..	..	..	2	..	..										
F	2	1	..	1	..	1	1	..	..										
G	2	..	..	..	..	..	2	..	1										

*General Remarks on Table III.*

The plant lived indefinitely in solutions K, A, B, C, and D, of all concentrations, and also in the lower concentrations of E, F, G, and H. But the higher pressures, when caused by the single salts, usually resulted in more or less speedy death. With a pressure of  $2892 N \times 10^{-4}$ , it was quite a common thing to find the cells dead within a week. Sometimes they were perceptibly

plasmolyzed before death ensued, sometimes not. Of the four simple solutions one appeared to affect the plant as much as another. The table shows the general result of the experiments almost graphically. In the higher concentrations the larger numbers appear mainly in the middle of the series, in the columns headed *Many*, while the third and eighth columns are nearly vacant. Passing to higher concentrations this gradually changes, until, in the highest, the reverse is true. The following conclusions may be drawn from these data.

1. *Nature of the stimulus.*—The effect of a solution of any given osmotic pressure upon the plant is both qualitatively and quantitatively the same, no matter what the chemical nature of the solution may be. If this effect were due to change in the amount of any one salt, then this fact would have appeared in the course of the experimentation, for my various solutions were devised to test this point. So we have eliminated, as far as the palmella form is concerned, the possibilities designated under 1, *a, b, c*, and 2, *b*, page 295. The stimulus cannot be of a chemical nature, nor can it be physical and depend more upon the presence of one salt than upon another. Also, we have eliminated 3 from among the possible primary stimuli. Considering the facts shown, it is impossible that the external stimulus should be either physical, with the necessary accompaniment of certain chemical factors, or yet chemical, with the necessary accompaniment of certain physical ones. It is, however, not only possible but probable that the external and primary physical stimulus may cause an internal and secondary chemical one. But that lies beyond the scope of the present research. Thus we are driven to accept 2, *a* as the truth in the matter, and to conclude that the response of the organism is determined by a physical, and never, as far as my experiments have gone, by a chemical stimulus. The effect of osmotic pressure upon the palmella form is a double one. I shall consider, first, the effect upon vegetative growth, and second, that upon reproduction.

2. *Response in vegetative growth.*—In my least concentrated solution (pressure  $96N \times 10^{-4}$ ) the mode of cell division almost

invariably changed. In forty-six cultures only three are exceptions to this. The cells at the edge of the original mass elongate and become ellipsoidal or even cylindrical, and when division occurs it is by means of a wall lying transverse to the long axis of the cell (*figs. 7, 15, 16*). Thus filaments grow out around the periphery of the original group. The interior cells do not change their form and do not divide further; at length they form the center of a mass of long radiating filaments. Branching of a cell occurs by means of a papilla-like lateral outgrowth, which is cut off by a wall transverse to its long axis. The walls between the filament cells thus formed do not split; thus there is no bulging of the end walls and no separating of the cells. The older cells of a filament (for division occurs only at the tip) bulge at the sides and become barrel-shaped, but the end walls hold them fast together and prevent them forming true spheres (*fig. 25*). It is apparently this coherence of the walls and the orientation of the walls with reference to the form of the cell which constitutes the filamentous habit.

As the osmotic pressure of the fluid is increased there is a continual lessening tendency on the part of the plant to produce filaments, and there arise fewer and fewer filaments at the edge of the cluster. Also these filaments tend to go back, by a rounding and breaking apart of their cells, into the original form. The older cells of the filaments do this, while the younger ones continue to advance by cylindrical cells (*figs. 4, 5, 10, 25, 31, 34*). In my most concentrated solution (pressure  $3856 N \times 10^{-4}$ ) there were still five cultures out of ten wherein filaments were formed, but in all but one of these the filaments were only two or three cells in length. Back of these cells a thick strand of palmelloid cells marked the path of the advancing filament tip (*figs. 20, 30*). This change of the cylindrical cell to a spherical one, accompanied, as it is, by a corresponding change in the direction of new walls, will be discussed when we consider the responses in filaments. For the present it is enough to note that with increasing pressure the tendency to produce peripheral filaments decreases, both in the number and in the length they attain

before reverting to the palmella form. In my weakest solution they often attained a length of twenty-five to thirty cells (but the older cells here are destroyed in the act of producing zoospores, which will presently be discussed), while in my strongest solution the few filaments produced seldom attained a length of over two cells. The tips of filaments are quite sharply pointed in weak solutions, but blunt in strong ones.

A maximum pressure beyond which filament growth cannot take place lies somewhere between  $1928N \times 10^{-4}$  and  $2892N \times 10^{-4}$ . No limit was found beyond which no filaments at all could be formed.

3. *Response in reproduction.*—The most marked response of this form to the change in osmotic pressure conditions is the sudden production of enormous numbers of zoospores. In some of my cultures almost every original cell has thus emptied out its contents within ten or twelve days of the date of transference. In general, within a fortnight and often within five days these very weak solutions become literally swarming with zoospores, and their production continues as long as the pressure remains low. Zoospores come both from original spherical or parenchyma-like cells and from the older cells of filaments radiating from the palmella mass. This method of producing zoospores has often been resorted to by various writers, and Klebs especially has found it successful in a great variety of forms. But, as far as I know, previous writers have not tried to determine the real nature of this weak solution stimulus.

As would be expected from the response in vegetative growth, when zoospores come to rest in these weak solutions and begin to enlarge, they do so by elongating into a cylinder, which soon divides transversely and later produces branches (*figs. 8, 14, 18, 32*). Thus the response to low osmotic pressure of a zoospore which has come to rest is the same as that of a young cell of the palmella form. Zoospores often grow at both ends, however, which I have not observed in case of other cells. The two cells formed by the first division of the zoospores thus sometimes become a base from which reach out two long branching filaments (*figs. 2, 18*).

In solutions of greater pressure zoospores are produced more and more tardily and in fewer and fewer numbers. The limit to the healthy production of zoospores lies at a pressure of about  $964 N \times 10^{-4}$ . Their production in a higher pressure is exceptional and I have never seen them produced at all in my strongest solution. With a pressure of  $964 N \times 10^{-4}$  all zoospores germinate very soon in the manner already described; some of them go directly into the palmella form while most of them germinate to produce filaments of two to five cells, and then these pass into the palmella condition by rounding and breaking up (*figs. 17, 19*). Thus it often comes about that cultures with this pressure show both forms growing together, short young filaments and free round cells as well as parenchyma-like masses. With pressures above the observed limit the spores, when they are produced, usually grow directly into the palmella form, and then continue to grow slowly, or go into the resting stage. I have given little attention to the fate of these resting spores. They retain their green color and remain indefinitely at the bottom of the culture dish. Klebs has described these and states that they may be made to germinate after complete drying out.

#### B. RESPONSES OF THE FILAMENTOUS FORM.

Experimental data from 169 transfers of filaments into the various solutions are presented in table IV, which is constructed on the same plan as table III. Whether or not the original material changed into the palmella form is shown in the third, fourth, and fifth columns. Cultures in which all or nearly all the cells went over into the other form within a period of thirty days are recorded in the third column; those in which some, but not all, went over, and long filaments persisted after twenty to forty days, are indicated in the fourth column. The fifth column records cultures in which the original filaments did not change in form but continued as such for twenty to forty days. The results as to the production of zoospores, whether many, few, or none, are tabulated under those respective heads. Where

zoospores were produced, but many or all of them did not germinate, the numbers are placed in the column headed *Ungerm.*

TABLE IV.

Pressure $96 N \times 10^{-4}$ or 16.19 cm. Hg.										Pressure $964 N \times 10^{-4}$ or 161.86 cm. Hg.									
Solution	Cultures	Palmella		Filaments	Spores				Died	Solution	Cultures	Palmella		Filaments	Spores				Died
		All	Few		Many	Few	Ungerm.	None				All	Few		Many	Few	Ungerm.	None	
K	6	..	..	6	6	..	..	..	..	K	7	3	1	2	7	..	6	..	..
A	6	..	..	6	6	..	..	..	..	A	6	1	4	1	6	..	3	..	..
B	6	..	..	6	6	..	..	..	..	B	6	4	..	2	6	..	3	..	..
C	6	..	..	5	5	..	2	..	..	C	6	3	3	..	3	3	3	..	..
D	6	..	..	5	5	..	..	..	1	D	6	2	3	1	6	..	3	..	..
E	7	..	1	6	5	2	..	..	1	E	6	..	4	2	2	3	2	1	2
F	1	..	..	1	1	..	..	..	..	F	1	..	1	..	..	..	..	1	..
G	1	..	..	1	1	..	..	..	..	G	1	..	1	..	..	1	1	..	..
H	1	..	..	1	1	..	..	..	..	H	1	..	1	..	1	..	..	..	..

Pressure $193 N \times 10^{-4}$ or 32.37 cm. Hg.										Pressure $1928 N \times 10^{-4}$ or 323.71 cm. Hg.									
Solution	Cultures	Palmella		Filaments	Spores				Died	Solution	Cultures	Palmella		Filaments	Spores				Died
		All	Few		Many	Few	Ungerm.	None				All	Few		Many	Few	Ungerm.	None	
K	3	..	..	3	3	..	3	..	..	K	6	3	2	1	2	4	4	..	..
A	3	..	3	..	2	1	2	..	..	A	5	3	1	1	1	2	1	2	..
B	3	..	..	3	3	..	3	..	..	B	5	5	..	..	2	..	2	3	..
C	3	..	..	3	3	..	3	..	..	C	5	5	..	..	2	3	3	..	..
D	3	..	..	3	3	..	..	..	..	D	5	3	..	..	..	3	1	..	2
E	2	..	..	2	2	..	..	..	..	E	5	..	2	..	..	..	..	2	3

Pressure $482 N \times 10^{-4}$ or 81.27 cm. Hg.										Pressure $2892 N \times 10^{-4}$ or 487.59 cm. Hg.									
Solution	Cultures	Palmella		Filaments	Spores				Died	Solution	Cultures	Palmella		Filaments	Spores				Died
		All	Few		Many	Few	Ungerm.	None				All	Few		Many	Few	Ungerm.	None	
K	4	..	1	3	..	..	3	..	..	K	1	1	..	..	..	..	1	..	..
A	3	3	..	..	3	..	3	..	..	A	3	2	1	..	..	1	1	2	..
B	3	3	..	..	3	..	3	..	..	B	3	3	..	..	..	2	..	1	..
C	3	3	..	..	3	..	3	..	..	C	3	2	1	..	..	2	2	1	..
D	3	..	3	..	3	..	3	..	..	D	3	2	1	..	..	..	3	..	..
E	3	..	2	1	1	..	2	..	..	E	6	1	..	..	..	..	2	5	..
										F	1	..	1	..	..	..	1	1	..
										G	1	..	..	..	..	..	..	1	..
										H	1	..	..	..	..	..	..	1	..

*General Remarks on Table IV.*

The only solutions in which death regularly ensued were the more concentrated ones made from single salts; *i e.*, E, F, G, H. But in almost every case where the original filaments died they evinced a great tendency to change into the palmella form

before death ensued. Often they did not die until they had actually taken the palmella form. Where a definite response was not observed before death, the cultures are recorded in the column headed *Died*. The graphic arrangement of the numbers in this table is even more striking than that in table III. The conclusions to be drawn from the above set of results will be given in the same order as were those derived from the experiments upon the palmella form.

1. *Nature of the stimulus*.—The stimulus with which we have to deal is always of an osmotic nature. Indeed, this generalization would have been expected from the nature of the stimulus affecting palmelloid cells, and from the data which we are now considering the same facts can be cited in its support.

2. *Response in vegetative growth*.—Filaments placed in the weakest solution used (pressure  $96 N \times 10^{-4}$ ) continued indefinitely to grow without change in form. No going over to palmella was observed until time enough had elapsed for great concentration of the solution through evaporation. The rule is, if filaments are left in a dish without renewal of fluid, they change to the other form at the end of a period of thirty to fifty days. By this time the culture is often nearly dry. Also, by addition of water from time to time filaments may be kept growing in a dish as long as one chooses. So there is little room for question as to what the nature of the stimulus producing this very tardy response really is. With higher osmotic pressures the ability of the alga to continue as filaments rapidly decreases, the first indication of such an effect being the blunting of the terminal cells. A limiting pressure for free filament growth lies apparently somewhere in the region between the pressure of  $193 N \times 10^{-4}$  and  $482 N \times 10^{-4}$ . With a pressure of  $964 N \times 10^{-4}$  only eight out of forty cultures showed good growing filaments after thirty days, while many of these cultures had lost all vestige of them within that time. Not a single one of twenty-two cultures having a pressure of  $2892 N \times 10^{-4}$  showed typical filaments at the end of such a period, and there were only four which showed filaments at all. As in the other form,

an absolute limit to the production of filaments was not found. A comparison of the limit found here with that found in the case of palmella material will be made later. Filaments floating on a strong solution change to the palmella form much more slowly than those at the bottom of the dish.

The morphological details of the response just discussed are as follows. If a filament be placed in a strong solution the oldest cells are the first to be visibly affected. They continue rounding up beyond the barrel-shaped stage and become nearly or quite spherical. This process is accompanied necessarily by a splitting of the common walls from their margins inward, until, when the cells have attained the spherical form, they are practically free from one another (*figs. 4, 5*). They may not break entirely apart, however, but may remain clinging together in loose strands. The limitation upon the direction of new walls (*cf.* discussion of the responses of palmella form, *p.* 300) is now apparently removed, and the cells proceed to divide in all directions. The new cells may separate entirely (*fig. 10*), may remain loosely attached to one another (*fig. 34*), or may form an irregular parenchyma-like colony (*figs. 6, 11*). In the latter case the rounding is of course incomplete, but such cells are always nearly isodiametric. Cell division goes on much more slowly in the palmella form than in the filamentous.

3. *Response in reproduction.*—The response is the same here as in the other forms and is equally marked. The pressure limit to the production of zoospores by the filamentous form is somewhat above  $964 N \times 10^{-4}$ . The limit to their germination into long filaments is below  $482 N \times 10^{-4}$ . Zoospores are produced here in the way already described. The length of a filament, even in a weak solution, is limited by the fact that its oldest cells are continually becoming zoosporangia and thus losing their contents (*fig. 16*).

#### General considerations.

The question which this research was undertaken to answer has been answered very clearly as far as the answer goes. The



stimulus to which the alga responds by a change of form is a change in the osmotic pressure of the surrounding fluid. This must be accompanied by a corresponding change in the relation of water to the protoplasm. Whether or not extraction of water brought about by solutions of non-electrolytes or by evaporation from a moist substratum will be accompanied by the same response as that brought about by a solution of electrolytes, my experimentation has not yet gone far enough to show. It is worthy of remark, however, that when found wild this alga was in the palmella form, and was growing in air on moist bark. Loeb<sup>8</sup> has recently brought about artificial parthenogenesis, both in the eggs of echinoderms and annelids by placing them in a solution which would extract water from them osmotically. The result was the same whether electrolytes or non-electrolytes were used. Thus in these eggs the phenomena of segmentation and growth are profoundly influenced by osmotic pressure. Whether or not his results have any connection with those given in the present paper is an open question. The recent work upon various grains and forage plants by Buffum and Slosson<sup>9</sup> furnishes data which may have a somewhat closer connection with my own. Absorption by seeds, their germination, and the growth of the plant were all greatly retarded in a strong solution and accelerated in a weak one. And this was true without regard to the chemical nature of the dissolved substance; a number of electrolytes were used and also non-electrolytes. On the other hand, my experiments have not been at all in accord with those of Copeland (*loc. cit.*) when he found, with peas, maize, and

<sup>8</sup> LOEB, J.: Further experiments on artificial parthenogenesis and the nature of the process of fertilization. *Amer. Jour. Physiol.* 4: 178. 1900.

———: Artificial parthenogenesis in annelids (*Chaetopterus*). *Science N. S.* 12: 170. 1900.

<sup>9</sup> (1) SLOSSON, E. E., and BUFFUM, B. C.: Alkali studies II, Bulletin 39, Wyoming Exp. Sta. 1898.

(2) BUFFUM, B. C.: Alkali studies III, Ninth Ann. Report, Wyoming Agric. Exp. Sta. 1899.

(3) SLOSSON, E. E.: Alkali studies IV, same. 1899.

(4) BUFFUM, B. C., and SLOSSON, E. E.: Alkali studies V, Tenth Ann. Report, Wyoming Agric. Exp. Sta. 1900.

buckwheat, that K-ions are osmotically more potent than any other ions in his nutrient solutions. Some erratic cultures in my series seem to support his statements, but no generalization can be made from them. Of interest, also, in this connection are the recent researches of Yasuda.<sup>10</sup> Working with infusoria, he finds that these organisms are able to adjust themselves to solutions of quite high concentration, and that, in general, the limit to their power of adjustment seems to be osmotically about the same, no matter what salts are used. In other words, the limit is apparently one of osmotic pressure. It is probable that many of the so-called chemical or nutritive effects of dissolved substances upon the plant organism may turn out, upon further investigation, to be wholly or in part osmotic.

In a weak solution vegetative growth is very much more rapid than in a strong one. This may be due to the fact that in a strong solution the water content of the protoplasm is reduced in amount below the limit for optimum lability. When the plant grows fastest and best, it is in the filamentous form. In the weak solution, where activity seems to be at a maximum, the ions of the electrolytes, which are essential for metabolism, are not plentiful. This may suggest how the cylindrical form of cell with its increased surface<sup>11</sup> may be advantageous. At any rate, we may be sure that the greater surface of the cylinder puts the plant into better condition for exchange of material with its surrounding medium. On the other hand, the more concentrated solution not only withholds water from the cells, but presents a demand upon them for water. The cell meets this in part by offering as small a surface as possible to the solution. In this case, although the requisite ions may be present, and even in the right number, the scarcity of water in the

<sup>10</sup> YASUDA, ATSUSHI: Studien über die Anpassungsfähigkeit einiger Infusorien an concentrirte Lösungen. Jour. Coll. Sci. Imp. Univ. Tokyo 13: 101-140. 1900.

<sup>11</sup> In a cylinder, the lateral surface is greater than that of a sphere of the same volume, as long as the ratio of the length to the diameter equals or exceeds 2.727. In typical filament cells of this alga the ratio of the diameters is 3, and it is often 4 and even greater. It is seldom less than 2.8. Thus it is shown that the filament cell offers more surface to the surrounding medium through its lateral walls alone, than does the palmella cell of equal volume.

protoplasm may so decrease the lability that rapid growth is impossible.

What may be the mechanics of this rounding up of cylindrical cells when placed in a concentrated solution is one of the most important problems suggested by the present research. The fact that the dead cellulose membrane is almost entirely reshaped during this process, without being dissolved, renders it probable that the change in form is directly caused by some turgor change within the cell. In a rounding cell the membrane moves and changes its form and, since it is entirely inert, the source of this motion must be either in the activity of the protoplasmic body itself, or it must be in the turgor pressure of the mass of liquid within. But since protoplasm and cellulose wall can be parted so readily during plasmolysis, the first alternative is well-nigh untenable. If the wall be forced into the spherical shape by a change in the pressure from within, this must be brought about by a change in the mass of the contained liquids. Now, this slight change in mass which might produce a change in the turgor of the cell is most probably due to an alteration in the amount of cell sap within the vacuole. When the surrounding medium suffers change in concentration, a change in the volume of the vacuole may come about through the protoplasmic sac, either secreting liquid or acting merely as a semipermeable membrane.

When filaments are placed in a concentrated solution their behavior suggests at once partial plasmolysis. Water may be extracted, the turgor pressure on the walls may be decreased, and by the forces of surface tension and cohesion the protoplasm may tend to round itself up into a sphere. If this be true, we have an explanation of the lateral bulging which accompanies the longitudinal shrinking of the cellulose envelope. If the protoplasm tended to assume a spherical form within the cylindrical wall, the pressure upon this would be decreased first at the angles. At the same time it would be relatively increased upon the lateral walls near their middle. Thus would come about a bulging of the lateral walls outward, and hence a shortening of

the cell and a drawing of the end walls towards each other. But the internal pressure is to be counted as almost nothing at the angles, while it is still considerable in the middle of each end wall. So the margins of the end walls would approach the middle of the cell more rapidly than do their central portions, and splitting of the common membrane of two adjacent cells would necessarily ensue. Several facts were observed in the cultures which seem to support some such hypothesis as the one just stated. I have placed filaments in a solution where they were completely plasmolyzed and killed without any change in form. In solutions a little less concentrated they are not plasmolyzed but round up rapidly and soon die, often in the palmella condition. With a still lower pressure the filament cells round up more slowly and live. Another fact suggesting this idea is that floating filaments can resist a stronger solution, and can resist it longer, than sunken ones. The former are to some extent in contact with the air, and thus present less surface than the latter to the liquid. Still another observation bearing upon this hypothesis of partial plasmolysis is that cylindrical cells are the only ones which are able to change their form after they have become mature. A spherical cell must remain so till it divides, even if it be in a solution of very low pressure. Only two other observations with which I am acquainted bear upon this question: Yasuda (*loc. cit.*) says infusoria in concentrated solutions tend to approach the spherical form, and Klebs (*loc. cit.*) notes that the form of *Stigeoclonium tenue* with which he was working had a tendency to produce round cells in a concentrated solution. The very marked response in the orientation of the planes in segmentation may be traceable to the change in the form of the cell, or it may not. The observed fact is that vegetative cells under low osmotic pressure divide only across their long axis (across the axis of a lateral outgrowth in the case of a branch), while under the influence of high osmotic pressures the cells divide in all directions. It is remarkable, too, that the stimulus determining the position of walls in vegetative division also brings about the extreme segmentation which occurs in the

zoosporangium. Perhaps this fact is attributable to the increased general activity in weak solutions. It has no relation to the form of the cell, since zoospores are produced from both spherical and cylindrical cells, as well as from those of intermediate shape.

My approximations to the pressure limits for the different responses are given in table V. The numbers are all coefficients of  $N \times 10^{-4}$ .

TABLE V.

	Maximum limit to free production of filaments	Maximum limit to production of any long filaments	Minimum limit to free production of palmella form	Minimum limit to production of any pal- melloid cells	Maximum limit to free production of zoospores	Maximum limit to any production of zoospores	Maximum limit to free germination of zoospores
From palmella	1928	2892	2892	964	964	1928	964
From filaments	193	482	1928	482	1928	2892	193

From table V it will be observed that with the exception of the limits for zoospore production, all are much higher for the palmella form than for the filamentous. Perhaps the palmella cells become attuned to conditions of high pressure, so that after they have existed under these conditions for some time they are enabled to form filaments, and maintain them, at a higher pressure than was possible previously. Buffum and Slosson (*loc. cit.*, 3) state that there was a continual increase in the salt content of seeds which were imbibing water from a salt solution. Thus the difference between internal and external pressures in the case of seeds is being gradually decreased, and this means that the effect of the external pressure in retarding absorption of water is gradually diminishing also. Now if this be true in these algal cells, we may have in it an explanation of the fact that the palmella form (always growing in concentrated solutions) becomes to a greater or less extent attuned to the effects of high pressure; the ratio of internal to external pressure may be nearer unity for a palmelloid cell than for a filamentous one. That the nature of the protoplasm itself is changed by prolonged subjection to the

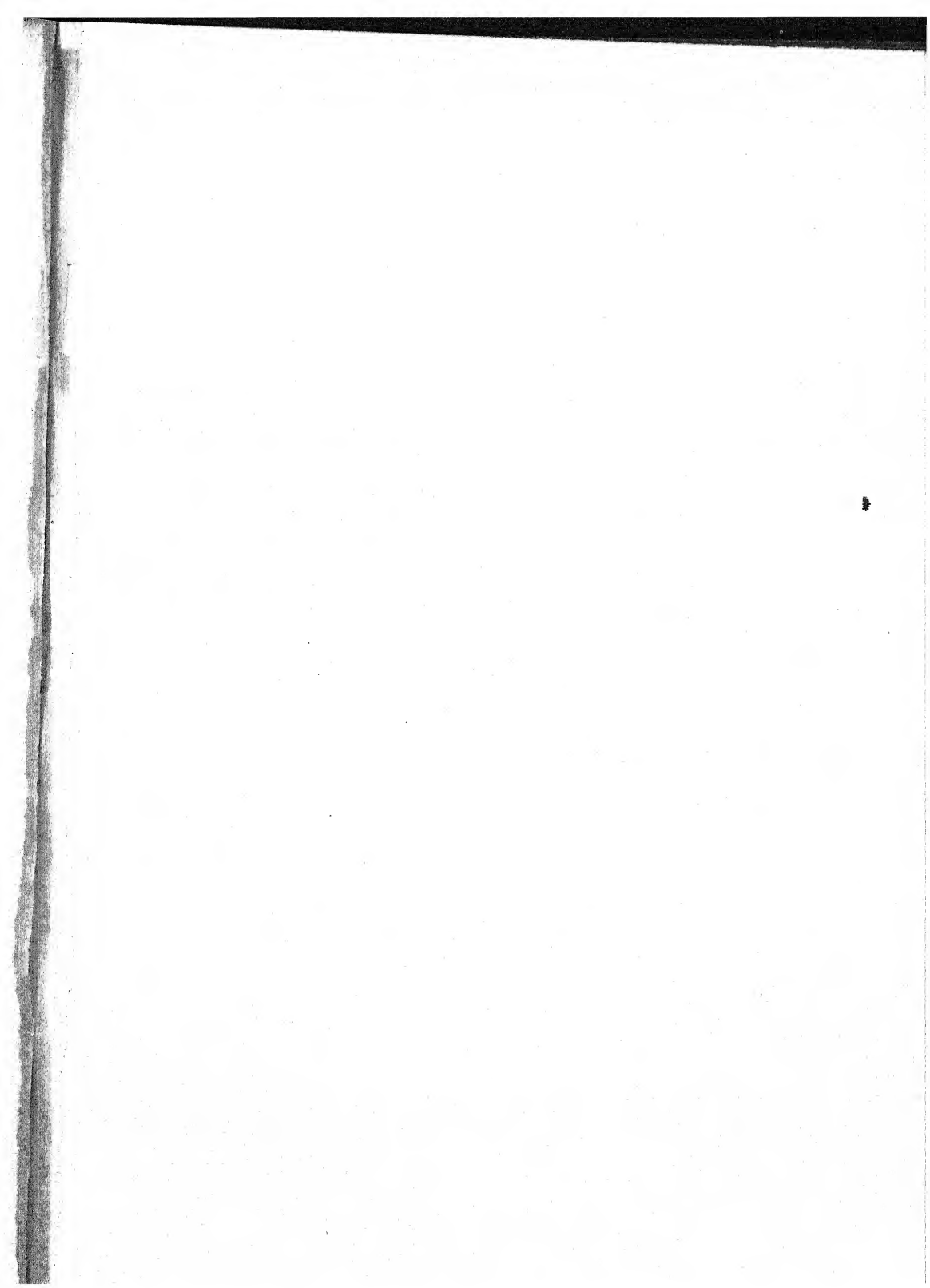
action of a concentrated solution is indicated by a comparison of the maximum concentrations for zoospore germination in the two forms. Zoospores from a palmelloid cell are able to produce and maintain filaments at a much higher pressure than can those produced from a filament. The two kinds of zoospores are physically alike, but are physiologically very different. It needs to be said here that in cases where the tables record ungerminated zoospores there are also many which did germinate to form filaments of several cells. In cultures so marked, however, the majority of zoospores failed to germinate in that way.

#### Summary.

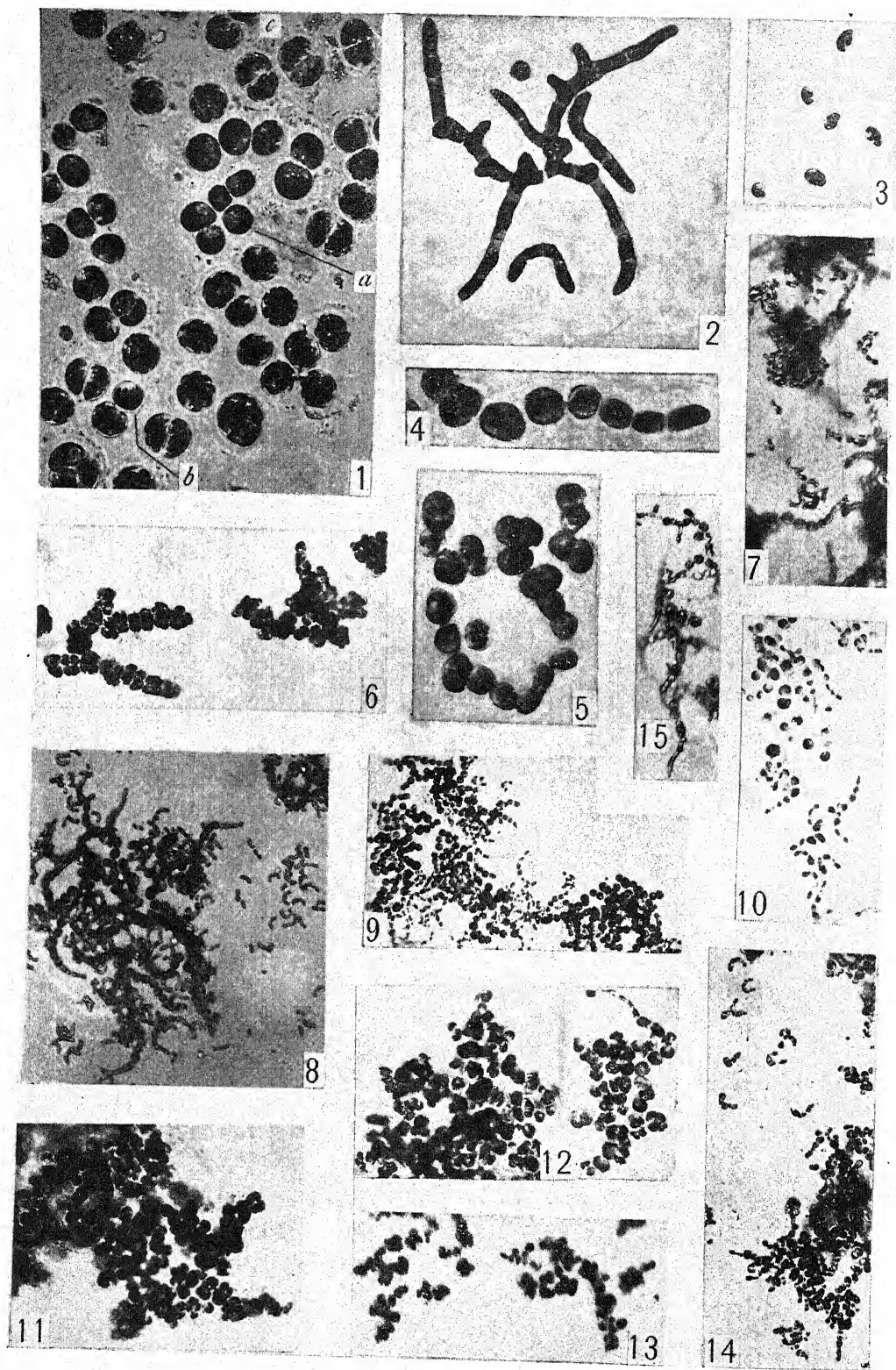
My results are as follows:

1. The responses of *Stigeoclonium* (*tenuis* ?), both in form and in reproductive activity, which accompany a change in concentration of the Knop's solution in which it is growing, are due to changes in the osmotic pressure of the medium, and are in no way functions of its chemical composition.
2. A high osmotic pressure affects the plant in four ways: (*a*) it decreases vegetative activity; (*b*) it inhibits the production of zoospores; (*c*) it causes cylindrical cells to become spherical; (*d*) it frees the alga from certain limitations as to the orientation of planes of cell division.
3. A low pressure has the following effects: (*a*) it increases vegetative activity; (*b*) it accelerates the production of zoospores; (*c*) it causes forming cells to become cylindrical; (*d*) it determines the orientation of the planes of cell division.
4. A zoospore which has come to rest responds in the same way as a palmelloid cell.
5. Cells of the palmella form become slightly attuned to the high external pressures of concentrated solutions and exhibit some responses quantitatively different from those of filaments.

In closing I wish to express my thanks to Professor F. C. Newcombe, of the University of Michigan, for much valuable suggestion and advice in the inception of the work, and to

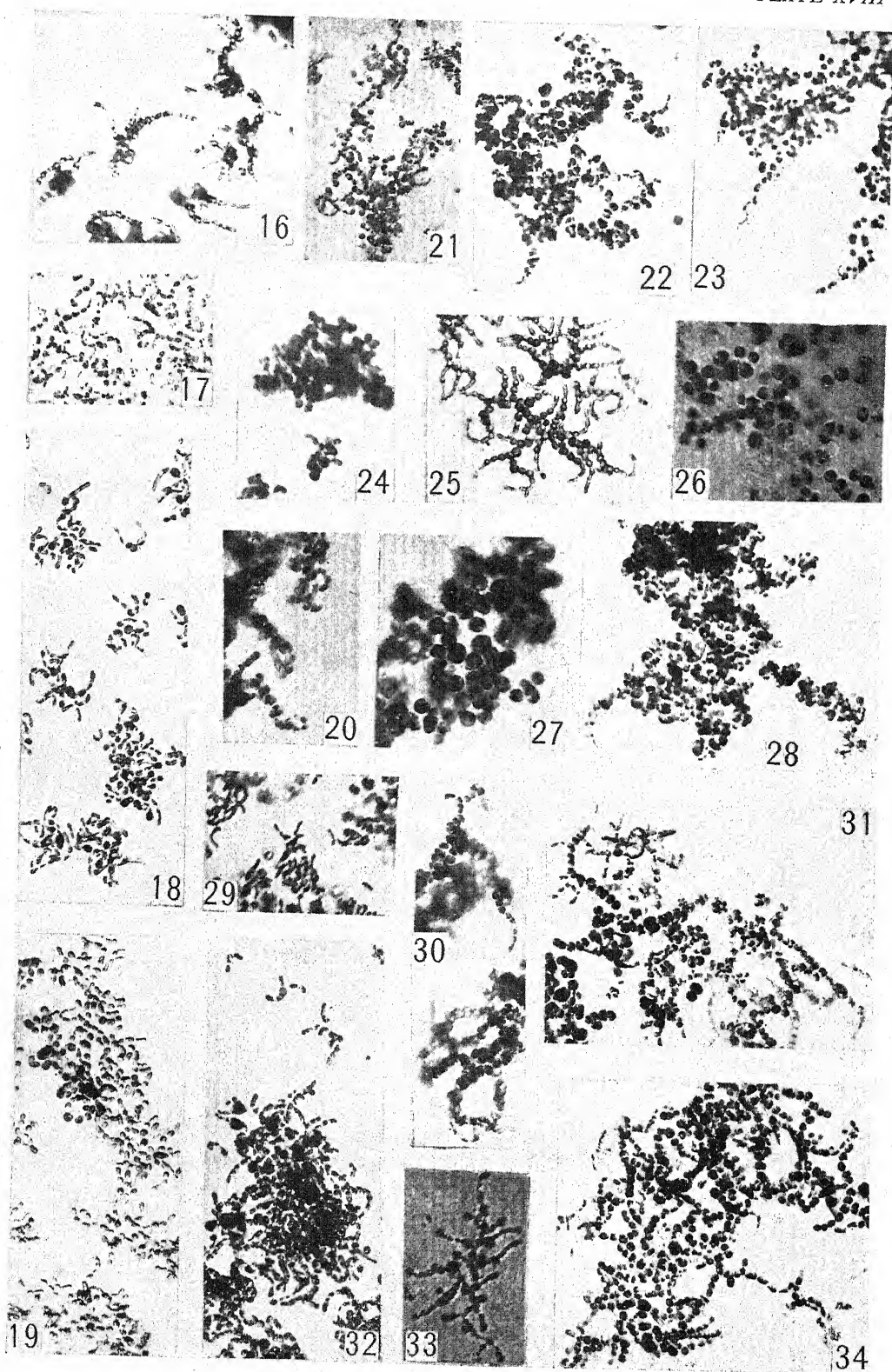






LIVINGSTON on STIGEOCLONIUM





LIVINGSTON on STIGEOCLONIUM



Professor C. R. Barnes, of the University of Chicago, for aid in its completion. Also, I take occasion here to acknowledge with thanks much kind assistance regarding physical chemistry rendered me by Dr. K. E. Guthe, of the University of Michigan.

THE UNIVERSITY OF CHICAGO.

#### EXPLANATION OF PLATES XVII AND XVIII.

All of the figures are reproductions of photomicrographs taken with a Zeiss camera and Zeiss microscope. The first five are magnified about 450 diameters, the rest only about 140. All but *figs. 1-5* were taken as the plants lay in or upon the nutrient medium in the culture dish. The others were made from slide mounts in water.

##### PLATE XVII.

FIG. 1. Typical palmella form. *a.* Vegetative cell, showing clear area not covered by chloroplast. *b.* The same, but preparing to produce zoospores. *c.* Cells which have divided, but whose daughter cells are not yet separated.

FIG. 2. Typical filaments.

FIG. 3. Zoospores, immediately after they have come to rest. The clear area is the part of the periphery not bounded by the chloroplast.

FIGS. 4 and 5. Typical filaments changing to the palmella form by rounding and separation of cells.

FIG. 6. Culture no. 50. Exposure May 10. Typical palmella material. The mass at the right shows a parenchymatous colony. This was used for original material in many of the following cultures.

FIG. 7. Culture no. 144*a*. Palmella from no. 50 put into K, 96  $N \times 10^{-4}$  May 4, 1900. Exposure May 28. Original palmella masses with long filaments at periphery. Also young filaments from zoospores. The original masses are out of focus.

FIG. 8. Culture no. 147*a*. Palmella from no. 50 put into C, 96  $N \times 10^{-4}$  May 7, 1900. Exposure May 28. Original masses have grown out into long filaments whose older cells have rounded. Many young filaments from zoospores.

FIG. 9. Culture no. 132*a*. Palmella from no. 50 put into K, 1928  $N \times 10^{-4}$  May 7, 1900. On May 23 many new filaments had been produced from zoospores but were rapidly breaking up. Exposure June 12. None of the original material is shown, the new filaments are almost entirely palmella. Compare this with *fig. 12*, which is from the same culture, exposure on the same day, but shows the original material. It has multiplied much, and one filament is shown reaching out from the edge of the old colony.

FIG. 10. Culture no. 141*b*. Palmella from no. 50 put into C, 964  $N \times$

10<sup>-4</sup> May 7, 1900. Exposure June 12. Filaments have grown from the original palmelloid cells but these break up as soon as the cells become a few days old. Original material is seen at the upper left.

FIG. 11. Culture no. 126*a*. Palmella from no. 50 put into K, 2892 *N* × 10<sup>-4</sup>, May 7, 1900. Exposure May 28. Typical palmella form.

FIG. 12. See explanation of *fig. 9*.

FIG. 13. Culture no. 128*a*. Palmella from no. 50 put into B, 2892 *N* × 10<sup>-4</sup> May 7, 1900. Exposure May 28. Typical palmella form. Material moved slightly during exposure, but its form is well shown.

FIGS. 14 and 15. Cultures no. 145*a* and 145*b*. Both these cultures palmella from no. 50 put into A, 96 *N* × 10<sup>-4</sup> May 7, 1900. Exposure May 28. Filaments radiate from the original colonies and many new filaments have been formed from zoospores. In *fig. 14* the material was floating, in *fig. 15* it was resting on the bottom of the dish. The latter shows no filaments from zoospores. They usually float on the surface.

FIG. 16. Culture no. 149*b*. Palmella from no. 50 put into E, 76 *N* × 10<sup>-4</sup> May 7, 1900. May 23 many new filaments had been formed both at edges of original masses and by zoospores. Exposure May 28. At that time the filaments from zoospores were long and their older cells were rounding up. These are shown. Zoospores and young filaments were still plentiful but did not occur in the field where the exposure was made. Many of the older cells are empty, having produced zoospores.

FIG. 17. Culture no. 139*a*. Palmella from no. 50, put into A, 964 *N* × 10<sup>-4</sup> May 7, 1900. Exposure June 16. Zoospores have been formed in large numbers and many have germinated, but they break apart and form palmelloid cells almost immediately. There are no filaments here more than three or four cells in length. Some resting spores which will not germinate are shown.

FIG. 18. Culture no. 148*a*. Palmella from no. 50, put into D, 96 *N* × 10<sup>-4</sup> May 7, 1900. Exposure May 26. Shows typical weak solution response. Surface covered with filaments produced from zoospores.

FIG. 19. Culture no. 140*a*. Palmella from no. 50, put into B, 964 *N* × 10<sup>-4</sup> May 7, 1900. Exposure June 15. Many zoospores were produced and germinated but went almost immediately into the palmella form. Figure shows filaments, palmella and some germinating spores.

FIG. 20. Culture no. 130*a*. Palmella from no. 50, put into D, 2892 *N* × 10<sup>-4</sup> May 7, 1900. Exposure May 28. Palmella still with scarcely any tendency to form filaments at edges.

FIG. 21. Culture no. 125*a*. Palmella from no. 50, put into E, 96 *N* × 10<sup>-4</sup> May 8, 1900. Exposure June 15. Long filaments have been produced from zoospores. Some of their older cells are becoming rounded. Many zoospores not yet germinated are seen. Most of these germinated later.

FIG. 22. Culture no. 108*a*. Filaments from no. 83*a* (whose appearance

was exactly that shown in *fig. 18*), put into K, 1928  $N \times 10^{-4}$  May 8, 1900. Exposure June 15. Very few and very short filaments at the edges of large palmella masses.

FIG. 23. Culture no. 109. Filaments from no. 83*a*, put into A, 1928  $N \times 10^{-4}$  May 8, 1900. Exposure June 15. Very few filaments at the edges of large palmella colonies.

FIG. 24. Culture no. 105*a*. Filaments from no. 83*a*, put into C, 2892  $N \times 10^{-4}$  May 8, 1900. Exposure May 28. Palmella with scarcely any tendency to form filaments at edges.

FIG. 25. Culture no. 118*b*. Filaments from no. 83*a*, put into D, 964  $N \times 10^{-4}$  May 8, 1900. Exposure June 15. Filaments were produced from zoospores and have grown long. Their older cells have rounded and the very oldest ones are truly palmella.

FIG. 26. Culture no. 176*a*. Filaments from no. 148*a* (*fig. 18*) put into K, 2892  $N \times 10^{-4}$  May 25, 1900. Exposure June 15. Nothing but palmella.

FIG. 27. Culture no. 111*b*. Filaments from no. 83*a*, put into C, 1928  $N \times 10^{-4}$  May 8, 1900. Exposure June 15. Nothing but palmella.

FIG. 28. Culture no. 116*a*. Filaments from no. 83*a*, put into B, 964  $N \times 10^{-4}$  May 8, 1900. Exposure June 15. Very few filaments at periphery of irregular masses of palmella.

FIG. 29. Culture no. 169*a*. Filaments from No. 148*a*, put into C, 964  $N \times 10^{-4}$  May 25, 1900. Exposure June 15. A mixed culture, showing palmella, young and old filaments, the latter changing to palmella, and zoospores. The figure is poor. True palmella is shown at the upper right.

FIG. 30. Culture no. 106*a*. Filaments from no. 83*a*, put into D, 2892  $N \times 10^{-4}$  May 8, 1900. Exposure May 28. Original filaments are traced by knotted strands of palmella. At the tip of each strand are two or three filament cells.

FIG. 31. Culture no. 123*a*. Filaments from no. 83*a*, put into C, 96  $N \times 10^{-4}$  May 8, 1900. Exposure June 15. Many new filaments were produced from zoospores. The original material has, in good part, gone to palmella by increased concentration from evaporation. The younger filaments continue filaments still, but their older cells become spherical.

FIG. 32. Culture no. 120*a*. Filaments from no. 83*a*, put into K, 96  $N \times 10^{-4}$  May 8, 1900. Exposure May 28. Original material growing as filaments. No true palmella. Also many young filaments and zoospores.

FIG. 33. Culture no. 122*a*. Filaments from no. 83*a*, put into B, 96  $N \times 10^{-4}$  May 8, 1900. Exposure May 28. Original material still growing finely as filaments. Young filaments and zoospores.

FIG. 34. Culture no. 158*a*. Filaments from No. 148*a*, put into H, 964  $N \times 10^{-4}$  May 25, 1900. Exposure June 15. There are scarcely any good filament cells. The palmella cells lie in loose rows, marking the direction of the original filaments.

## OBSERVATIONS ON LESSONIA.

CONWAY MACMILLAN.

(WITH PLATES XIX-XXI)

THE observations recorded in this paper have been made upon a plant cast up upon the beach of Vancouver island at Baird point, on the strait of Juan de Fuca. It was collected by Miss Josephine E. Tilden, August 3, 1898, and portions of it were distributed in her *American Algae* under the name of *Lessonia littoralis* Farlow & Setchell, this determination having been made by Professor De Alton Saunders and kindly furnished us by him. The plant when collected was apparently quite fresh, and must have been developed near the point where it was found. The day before its collection, I am informed by Miss Tilden that heavy seas had been coming in, and these no doubt dislodged the plant and carried it up upon the beach. From the shape of the holdfast it must have been growing in a cup-shaped depression of the rocks from which it had been detached without great difficulty.

The general habit and appearance of the specimen is shown in *plate XIX*, taken from a photograph made under my direction by Mr. C. J. Hibbard, of Minneapolis. The plant was removed from its tank of formalose and spread out upon a table top, which was then tilted at an angle of  $50^{\circ}$ , and the camera was lifted close to the ceiling of the room, and depressed so that the view is not appreciably foreshortened and gives a fair idea of the plant. From the holdfast to the tips of the longest laminae this specimen measured two meters in length. At the base the holdfast measures 9<sup>cm</sup> in diameter. Immediately above the holdfast area the stipe is irregularly branched, developing seven principal trunks, each of which soon branches again. Owing to its probable confinement in an almost hemispherical depression, the holdfast is extremely condensed and contorted. Viewed from

below it presents in places an almost smooth fixation area, rounded off and mottled by the superposition of and compression together of the different hapteric branches. This generally bulbous base is irregular on one side, where a protruding corner of rock had entered it, and is hollow, indicating that many of the hapteric branches were turned under the base of the stem.

The main stipe can scarcely be distinguished from the holdfast by which its lower portion has been overgrown. Of the seven branches which arise the largest is 3.5<sup>cm</sup> in diameter, while the smallest is 6<sup>mm</sup>. All of these appear to be about the same age, and all are strongly fenestrate near the base. This, I presume, may be due to the growth of epiphytic animals and vegetation. The whole base of the plant is covered with a growth of bryozoans, together with *Cladophora*, *Rhodomenia*, and other algæ, while barnacles and sertularian hydroid colonies are grown in the lacunæ. It is to the growth of some of these organisms that I am inclined to attribute the lattice-like and hollow character of the stipe area near the base. Similar pathological perforations and lacunæ are found on distal regions, but not so abundantly. For the most part the more distal portions of the stipe are solid, as in the tribe *Lessoniæ* generally. Toward the base the stipe is cylindrical, but it becomes flattened distally. Thus, 20<sup>cm</sup> above the holdfast, cylindrical branches 12<sup>mm</sup> in diameter are developed. Along the same branch system 10<sup>cm</sup> distally, the stipe is 12<sup>mm</sup> by 8<sup>mm</sup> in transection. Distally 8<sup>cm</sup> of this, on the same branch system, the flattening is stronger, and sections 15<sup>mm</sup> by 4<sup>mm</sup> are found, while the terminal portions of the stipe which give rise to the separate laminae may be but 2 or 3<sup>mm</sup> in thickness and 20<sup>mm</sup> in breadth. The branching throughout is dichotomous, and the paired laminae are ordinarily produced in groups of four at the tips of the flattened stipe. Each lamina has what may be called a petiolate base, and this is sometimes cylindrical in cross section, though more often flattened. The general color of the stipe and holdfast is brown, and with slight change the tint persists beyond the petioles of the laminae. The laminae themselves are lighter in tint, becoming quite green



in the formalose preparation, but not so different in color in the fresh material.

The laminae, of which over eight hundred are present upon the specimen under observation, vary in length from 10<sup>cm</sup> on weaker lateral branches to over a meter on the stronger and central branches. They are all ribbon-shaped, thin, and gradually attenuated to a point. Robust and well-developed laminae measure from 5-6<sup>cm</sup> across near the base, but many of them are scarcely 1<sup>cm</sup> in diameter. Some of the leaves have very well-marked midribs, while others are quite devoid of them, and it is the narrow leaves which are provided most constantly with midribs, while the broader leaves commonly do not develop them. Through the dichotomy of the plant it generally happens that two pairs of leaves are borne close together; that is the laminae appear in groups of four, of which two may be described as inner and the other two as outer. All four of the leaves may be of the slender type with midribs, or the two outer leaves may be broad and the two inner narrow. So far as this specimen shows, it is only upon the broad leaves without midribs that sori of sporangia are produced. When the midrib is present it is often very wide, reaching within 2<sup>mm</sup> of the leaf-margin, but in many of the slenderer leaves the midrib is only one third the diameter of the whole lamina. There is a difference, too, between the broad and narrow leaves in their bases, the broad ones being often rounded, while the narrow ones are attenuate. Sori, when present, form patches 10<sup>cm</sup> or more in length on both sides of the broad-based sporophylls. Two or three such sori are found in succession upon larger sporangium-bearing laminae, and give a somewhat blistered appearance to the leaf, owing to the characteristic loosening of the cuticle common to all Laminariaceæ.

The presence of two kinds of leaves has been already noted in other members of the genus, and the explanation given in some instances has been that the leaves are biennial. During the first year they fail to produce sporangia, but later, during the second year of their life, sporangia are produced upon them.



This is the explanation given by Areschoug<sup>1</sup> of the leaves of *Lessonia nigrescens* Bory. In *Lessonia fuscescens* Bory the older leaves are two-horned at the apex, this being caused by the carrying away of the upper part when decayed, as described by Hooker and Harvey.<sup>2</sup> In this species the sorus itself falls away from the frond and the old leaves must present a very different appearance from the younger ones. In *Lessonia littoralis* the larger leaves have lost their tips, but this does not seem to be due to a sloughing off of the sori, since the position of the latter is basal. The position of the two kinds of leaves in the species under consideration makes it somewhat difficult to believe that their difference is purely a matter of age. Unfortunately young material is not at hand, and this, as well as a large number of other interesting questions, must be held in abeyance. It is true that the sporophylls are more coriaceous than the slender sterile leaves, and as will appear in the histological portion of this paper the general structure of the sporophyll is nearly equivalent to that of the midrib in sterile leaves. Nevertheless, from the very constant position of the leaves upon the ultimate branches of the stipe, it is difficult to assign them different ages unless some modification of the ordinary development by basal splitting has arisen. Indeed some evidence of this may be derived from the specimen under observation, in which very often but a single lamina of the narrow form appears in the notch between two of the broad laminae, and in a few instances the single central lamina is split at the base, thus giving it two petioles. It is therefore possible to conceive how by the continued growth of the plant the pair of leaves of one year may be separated by the pair of the succeeding year. This again is very much as described for *Lessonia nigrescens* by Areschoug in the work cited, and it may be said also of *Lessonia littoralis* with tolerable certainty that there is a "true defoliation and a true foliation."

If all laminae at the end of a branch could be regarded as a

<sup>1</sup>ARESCHOUG: Observ. Phyc. 5: 8. 1884.

<sup>2</sup>HOOKE and HARVEY: Flora Antarctica 2: 457. 1847.

unit and taken into the mind without reference to their different ages, a comparison might be instituted with such forms as *Alaria*, in which the sporophylls are lateral at the base of the main frond. In *Lessonia* the two middle younger laminae would represent the central portion of the generalized lamina, while the outer pair of sporophylls occupying a lateral position would represent the wings. Year after year, during the life of the plant, the process of basal splitting, exfoliation of the tip, and dichotomy of the stipe continues and there are constantly present groups of laminae typically in fours, the two inner representing the midrib portion (in which the basal split actually occurs), and the two outer essentially marginal areas of the hypothetical single lamina. Without developmental stages, comprising a series of young plants for comparison, it is scarcely possible to settle the points that have been raised.

The genus *Lessonia*, founded by Bory,<sup>3</sup> has not a very large literature. The most entertaining account of the remarkable plants classed under this genus is that of Hooker and Harvey in the *Flora Antarctica* already cited. Here is pictured very vividly the habit of the antarctic forms as they appeared to the young Hooker upon his cruise as assistant-surgeon of H. M. S. *Erebus*, under command of Captain Sir James Clark Ross.

This and the following are truly wonderful Algæ, whether seen in the water or on the beach; for they are arborescent, dichotomously branched trees, with the branches pendulous and again divided into sprays, from which hang linear leaves 1-3 feet long. The trunks usually are about 5-10 feet long, as thick as the human thigh, rather contracted at the very base, and again diminishing upwards. The individual plants are attached in groups or solitary, but gregarious, like the pine or oak, extending over a considerable surface, so as to form a miniature forest, which is entirely submerged during high-water or even half-tide, but whose topmost branches project above the surface at the ebb. To sail in a boat over these groves on a calm day affords the naturalist a delightful recreation; for he may there witness, in the antarctic regions, and below the surface of the ocean, as busy a scene as is presented by the coral reefs of the tropics.

Hooker goes on to say how the massive stems of *Lessonia fuscescens* have been mistaken by ship-masters for driftwood, and

<sup>3</sup> BORY: Duperry. Voy. Bot. Crypt. 1828.

how the Gauchoes use the cartilaginous stem for knife-handles; at the same time he gives, especially for *Lessonia ovata*, an extremely useful and accurate discussion of the morphology, and does not fail to point out several important histological facts upon which, in general, later accounts have been based. The most extensive anatomical study in the genus with which I am familiar is that of Grabendörfer,<sup>4</sup> whose results in a general way would apply to *Lessonia littoralis* as well as to the species of his investigation, *Lessonia ovata*. The figures given by him, five in number, illustrate but scantily the anatomical structure of the genus. I assume, also, that they were made from dried material, and the very different detail which I have found, and present in the accompanying plates, may be regarded as the result of studying material practically equivalent to fresh. The method of preserving large algæ in use at the University of Minnesota has not yet broken down in a single instance, although over a thousand jars of formalose material are on the shelves. Four per cent. solution of formalose is used, but after the first few months the jars are opened and fresh preservative substituted. After this it is our experience that disintegration does not occur.

Other literature which has been used in the study of this plant and has been more or less helpful is cited below.<sup>5</sup>

<sup>4</sup> GRABENDÖRFER: Beitr. zur Kenntniss der Tange. Bot. Zeit. 43: 641. 1885.

<sup>5</sup> KJELLMAN: Laminariaceæ in Engler and Prantl Pflanzenfamilien I. 2: 242. 1893.

WILLE: Ueber die Wanderung der anorganischen Nährstoffe bei den Laminariaceen. Festschrift für Schwendener. Berlin. 1899.

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WILLE: Zur Anatomie von *Macrocystis luxurians*. Bot. Zeit. 42: 801. 1884.

SETCHELL: On the classification and geographical distribution of the Laminariaceæ. Trans. Conn. Acad. 9: 333. 1893.

MACMILLAN: Observations on Nereocystis. Bull. Torr. Bot. Club 26: 273. 1899.

*Histology.*—The anatomical study of *Lessonia littoralis*, herewith presented, is based upon a series of slides made for me at my request by Mr. Harold L. Lyon, Instructor in the Botanical Department of the University of Minnesota. The material was all passed through the alcohols and embedded in paraffin in the usual way. The sections were cut upon a Minot precision microtome, and transferred gradually from clearing agents back to formalose water. Many of them were stained with aniline-water-safranin and aniline-blue, and permanent mounts in formalose water were prepared by sealing the cover slips at the edges. Mr. Lyon found that the ordinary mounting media distorted the sections, and after a few trials of other media he settled upon the formalose water as the best. I shall consider the different areas of the plant in order, beginning at the proximal regions.

*The holdfast.*—As has already been stated, the holdfast area of our specimen was much distorted and confused by the inextricable matting together and coalescence of originally separate branches. The primitive disk area was not definitely distinguished. Sections through the central and basal region of the holdfast showed a most confusing and irregular tissue, evidently the result of appression of hapteres to each other's surfaces. In cavities of the irregular mass of the holdfast some unattached hapteric branches were found, and these showed the ordinary dichotomous branching, cylindrial shape, and smooth green surface characteristic of the higher Laminariaceæ. They did not essentially differ in appearance from those of *Nereocystis*, described by me last year, nor from those of *Laminaria* previously described by Foslie and others. They measured less than 2<sup>mm</sup> in diameter in ultimate branches, and forked repeatedly within narrow vertical limits. Cross sections showed them to be composed of a pretty homogeneous thin-walled prosenchymatous tissue in which the cells are of varying sizes, averaging from 6–30 $\mu$  in diameter. Towards the surface the diameter of the cells lying in the radius of the cylinder is elongated, and there is a general superficial cambial area extending around the

holdfast and continuous over its tip immediately under the epidermis. The epidermal cells and the layer underneath are the only ones that take a protoplasmic stain with avidity, but the layers within these, to the number of five or six, contain numerous sharply staining chlorophyll bodies. It is apparent, therefore, that the growth in thickness of the holdfast is essentially superficial, and its whole tissue is produced from the cambial areas somewhat as is phelloderm from the cork cambium of higher plants. The epidermal cells proper are much the smallest of all those that make up the holdfast area, averaging from  $5-10\mu$  in height. There is no differentiated medullary area in the organ.

The longitudinal section of a haptere shows the customary structure, and serves to make clear that the growth in length takes place in the same way as does the growth in thickness. Toward the center of the section the cells are much elongated, running in some instances as high as one tenth of a millimeter in length. This length diminishes toward the tip of the organ, and directly under the tip a vertical section does not differ essentially in appearance from a transection. The dichotomous branching of the holdfast is due, as in *Nereocystis* and generally in *Laminariaceæ*, to the appearance of adjacent circular fields of cambium which undergo division more rapidly than the intervening and surrounding areas, so that the centers of these fields are protruded as the tips of the two new hapteric branches.

In older hapteres which have become matted together in the general holdfast mass, the walls are somewhat thickened in all the cells. Many of the cells are collapsed and distorted by pressure, and whole hapteric branches seem in places to be engulfed in the general tissue, reminding one of the appressions of hapteres to each other that often occur in other plants of the order. It does not seem probable that there is any rhythmical thickening of holdfasts or hapteres, such as takes place in the stipe of *Lessonia*, and produces the curious rings resembling those of an exogenous tree.

*The stipe.*—Very young stipe areas in *Lessonia* are much flattened, being morphologically nothing more than proximal areas of the lamina. As the plant grows, however, the region of the stipe undergoes a kind of secondary thickening, resulting in a ringed structure; and, by the greater thickness of the ring on one or both sides of the original flattened organ than over the edges, the whole stem changes from a flattened to a cylindrical body. The pith in all such cylindrical stems, even when they are very large, persists in its original thin and flattened structure, and does not appear in the form of cylindrical medulla as it does in *Nereocystis*. The pith is very often excentrically placed, and the number of rings of growth on one side may apparently be twice as many as on the other.

I have not found anywhere in the literature an adequate account of the anatomical basis of the phenomena which have been known and commented upon since the first discovery of *Lessoniæ*; and in the plates accompanying this paper an especial effort has been made to indicate the occasion for the ringed appearance so characteristic of the *Lessonia* stipe. It has long been suspected that the rings are in no sense annual rings, but it has been believed that they are connected with the development of the tufts of leaves in such a way that for each cycle of leaves there should be an additional ring of tissue developed in the older portions of the stipe. No doubt this view is correct, and from the exceedingly rapid growth of these gigantic algæ it is altogether reasonable to suppose that several rings might be produced in a single season.

Cross sections were made of a stipe 1<sup>cm</sup> in diameter and nearly cylindrical in form. In this the pith was placed excentrically and measured 5<sup>mm</sup> in length by 1<sup>mm</sup> in breadth, and lay 2<sup>mm</sup> from one side of the stem and 7<sup>mm</sup> from the other. Five distinct rings of growth were apparent on one side of the pith, and but three upon the other. The pith itself looks almost precisely the same in vertical section as in cross section, and is made up of an anastomosing web of loosely interlaced filaments imbedded in gelatin and filled with reserve food materials. The pith does

not stop abruptly where the inner ring of cortical tissue abuts upon it, but occasional medullary filaments run out between the thicker walled and more compactly placed filaments of the cortex. Trumpet hyphæ, abundantly represented in younger pith, are not so conspicuous in material of this age. The characteristic sieve-tubes of *Nereocystis* and *Macrocystis*, as described by Parker, Wille, Oliver, and others, have not been seen in *Lessonia*. The tissue immediately outside of the pith has in cross-section much the look of sclerenchyma. The cells in longitudinal section are not conspicuously armed, nor, as one looks at the cross-section under the microscope, do they seem to lie in radial rows. The inner ring on both sides of the pith is principally composed of such cells as have been described. The transition to the next outer ring is more clearly marked in the gross structure than under the microscope, and at first I was much puzzled because the thin sections which showed the rings very conspicuously when held up to the light did not show them clearly when placed under an objective. The occasion for this seems to be that cells of the new growth are at first somewhat more armed, and in places stand in rows extending in a radial direction and at the same level. The walls, except over the ends of the arms, are very thick. This has been shown both in diagram and in camera-lucida sections in *figs. 8-16*. Cross sections taken through a region where the armed-cell tissue contains such radial rows at the same level will here and there cut eight or more cells in such a way that the whole row comes into view looking somewhat like a medullary ray. Since the different radial rows are not all cut through the central portions of the cells, most of the tissue will preserve its sclerenchymatous appearance and the "medullary rays," so-called (they are of course *not* pith rays), come out as streaks of lighter appearance in the cross section. As the new ring of growth grows older the cells shorten their arms and become more condensed in appearance. This shortening of the arms, together with a less parallel arrangement, makes the light streaks shorter, narrower, and finally indistinguishable from the structure around them in the

section. The difference then between the inner and outer face of a ring of growth lies in the more armed shape of the inner cells, and the less armed shape of the outer, and the optical characters of the more-armed cells in the tissue are rendered peculiar by their tendency to stand in radial rows for some distance at the same level. In general the outer rings show longer rays and a generally coarser structure than do the inner, while the innermost ring of all around the pith shows the rays but sparingly, and in cross section is almost everywhere strongly sclerenchymatous in aspect. In the outer zones I have seen as many as nineteen cells in one of the rays, showing that this number of armed cells stood at exactly the same level in a radial plane and were all cut directly through the middle when the section was taken. A comparison of the cross and long sections as presented in the plate should make this matter clear. None of the zones of growth, except the one immediately around the pith, are free over any considerable area from the rays resulting in the manner described. Lying outside of the outermost zone will be found the immediate products of the superficial cambium, not yet modified into the characteristic armed-cells, but presenting a more isodiametrical character. The actual cambial region seems to lie immediately under the epidermis. Throughout the cortical region and extending down to the region of armed sclerenchymatous cells chloroplastids are abundant, and the epidermal cells themselves, with the layers immediately beneath, take dense protoplasmic stains. There is probably division of cells going on in several of the layers under the epidermis, but it would seem to be more active close to the epidermis than in the deeper layers. Three or four layers of cells immediately under the epidermis are of the same prismatic shape as the epidermal cells proper, and almost as broad as high. Deeper cells become elongated in a direction perpendicular to the epidermis, and their walls become thinner. In vertical section they are seen to lie in definite rows. The distinction made by Grabendörfer between outer and inner cortex in *Lessonia ovata* seems to hold good also for *Lessonia littoralis*, but there is a gradual transition



from one area to the other, and the most abrupt demarcation is that between the inner cortex and the pith. Indeed cells which begin life as components of the outer cortex may later become modified into components of the inner cortex, as the stem thickens.

Some peculiarities of structure were noticed, especially globose thickenings where the arms of cells came together in the ray-like rows of inner cortex cells. These bodies of cell-wall substance in their position and form remind one quite exactly of similar structures common in Rhodophyceæ and recently figured for *Gigartina* by Miss Olson.<sup>6</sup> The "Hohl-räume" of Grabendörfer I have not encountered in any of the sections under observation, but it is not improbable that they might exist in this species in older stipe areas. Granular cell contents are pretty abundant throughout the plant. The pith is particularly well supplied and the sclerenchymatous tissue of the inner cortex contains an abundance of granules.

In general it may be said of the older stipe of *Lessonia littoralis* that it consists of a strongly flattened pith surrounded by an inner cortex in which several zones of growth may be present, and an outer cortex consisting of a generally cambial group of cells arranged in from ten to twenty layers. Secondary thickening originates in the superficial cambial region, and the zonate appearance of a mature stem is due to the rhythmical production of more-armed and less-armed cells; but the difference between these is so slight that the optical distinction can scarcely be accounted for without taking into consideration also the peculiar juxtaposition of cells, especially in later zones, in radial rows at the same level.

*Transition to the lamina.*—The stipe becomes much flattened and the structure simplified in its distal portions. At the transition area, where the longitudinal rifts originate by which the laminae are separated from each other, the structure can be observed by sectioning the petiolate bases of laminae. These are sometimes nearly cylindrical, but generally more or less oval in cross section.

<sup>6</sup>Olson: Observations on *Gigartina*. Minn. Bot. Stud. 2: 154. 1899.

In this region the pith is large, composed of slender interlacing hyphæ, the majority of which seem to run transversely. Mingled with the ordinary hyphæ of the pith web are numerous trumpet hyphæ with the well-known sieve-plates, where the flared-out ends of the cells come in contact with each other. Very many of the trumpet hyphæ run transversely in the pith web, and they are perhaps rather less abundant in the perimedullary portion where they take principally a longitudinal direction. The interstices between the hyphæ are filled with gelatin, and immediately outside of the general mass of interlocking and interlacing filaments is a sheath of sclerenchyma, between the longitudinally extended cells of which occasional pith hyphæ extend transversely. The pith in this region occupies about one third of the diameter of the cross section. The remaining area is composed of three well-marked layers. Immediately outside of the pith is the narrow band of sclerenchyma just described, an area not staining deeply with aniline-water-safranin. Outside of this sclerenchyma lies a band of fundamental tissue having the appearance of parenchyma in cross section. The contents of the cells are not strongly granular. Outside of the last mentioned region is chlorenchymatous tissue, in which the cells show a marked tendency to develop with their long axes perpendicular to the epidermis. Surrounding the whole is a characteristic epidermal layer of low prismatic cells. The longitudinal section through this area does not show a development of the armed cells so abundant in older stipe and characteristic of the zones of secondary thickening, but the layer directly under the chlorenchyma is composed of almost isodiametrical cells rapidly changing in form to elongated-prosenchymatous in passing from the periphery towards the pith. The cells immediately around the pith are very long, some of them measuring  $1^{\text{mm}}$  from end to end, while the lumen as revealed in cross sections is sometimes not over  $2\mu$  in diameter. This condition reminds one of the similar shaped sieve-tubes of *Nereocystis*, which occur in the corresponding region of young stipe and lamina, but no end plates or callus has been detected in these cells of *Lessonia*, which in other

respects resemble the true sieve-tubes in form. There seems to be the same evidence of passive elongation and occasional obliteration that was noted in *Nereocystis*, and the same fragmentation of nuclei no doubt initiates the process of elongation. Upon the comparison of *Lessonia* with *Nereocystis*, it would seem reasonable to describe the "true sieve-tubes" of Wille and Oliver as modified sclerenchymatous elements of the perimedullary cortex.

The splitting of the lamina has not been definitely studied in this material, but so far as can be judged from the observations that have been made it takes place in the same manner as previously described for *Nereocystis*.

*The lamina.*—The essential structural basis of the lamina and the arrangement of the tissues indicate its complete morphological equivalence to the stipe. The pith is of course greatly extended into a narrow ribbon, the cells of which while retaining the web structure are not conspicuous for granular contents, indicating that the stipe serves in some sense as a reserve organ, while the leaf, essentially photosynthetic, uses its pith more particularly as strengthening tissue and a conduction path. On each side of the ribbon-shaped pith parenchymatous tissue is developed outside of a narrow intervening layer of sclerenchyma. Underneath the epidermis chlorenchyma cells extend, in from five to ten layers. The epidermal cells are not so low as those of the stipe, but generally show a long diameter perpendicular to the surface of the lamina. A well marked cuticle staining deeply is uniformly present. The edges of the lamina are occupied by cells (belonging in part to the pith area and in part to the cortex) with small diameter and thick walls, and these give strength to the margin. In young leaves it is probable that the midrib area is not immediately differentiated. The appearance of a midrib is caused simply by the greater thickness of the cortex along the central portion of the lamina and its somewhat abrupt thinning out at the sides. I was unable to distinguish in the pith tract any distinction between that portion which lay in the midrib and the portions on either side, nor do the cortical

cells differ in character in midrib and wings. The midrib may therefore be described in *Lessonia* as a central longitudinal hypertrophic cortex-area of the lamina.

*The structure and development of the sorus.*—The development of the sorus in *Lessonia littoralis* does not differ in any important respects from that described by many observers for other genera of the Laminariaceæ. The epidermal cells elongate into paraphyses, from the bases of which the sporangia arise. The sporangia themselves in some instances become almost as long as the paraphyses, reaching a length of  $45\mu$ , but this is unusual. Commonly they are from one half to two thirds as long as the slender paraphysal filaments, and of an elongated ellipsoid or club-like form. The zoospores are about  $4\mu$  in diameter, and have been observed in different stages of formation. A peculiar condition which I have not seen in other Laminariaceæ exists in the exfoliated cuticle. This during the extension of the epidermal cells into paraphyses has become greatly thickened, and may separate eventually after the manner described long ago by Thuret.<sup>7</sup> It does not always, however, in *Lessonia* separate as a continuous membrane, but is often broken up into pieces corresponding to the original epidermal cells and retaining a generally prismatic outline. Each paraphysis over a large area may carry on its end such a little cuticular cap.

The drawings of sections have been made by Miss Josephine E. Tilden, to whom I must express my thanks not only for this assistance, but for the specimen upon which my observations have been based.

THE UNIVERSITY OF MINNESOTA.

#### EXPLANATION OF PLATES XIX-XXI.

All sections not described as diagrammatic were drawn with a Zeiss camera lucida under a magnification of 600 diameters, and reduced one half in reproduction.

#### PLATE XIX.

*Lessonia littoralis*, from photograph.

<sup>7</sup>THURET: Ann. Sci. Nat. Bot. 1850.

## PLATE XX.

FIG. 1. Diagram of end of branch bearing two pairs of leaves, the outer pair without midribs and bearing sori, the inner with midribs and without sori.

FIG. 2. Cross section of haptere showing epidermis, superficial cambium, and ground tissue.

FIG. 3. Longitudinal section of haptere through superficial area.

FIG. 4. Longitudinal section through central portion of haptere showing elongated parenchymatous cells.

FIG. 5. Section through older area of holdfast showing irregular and distorted structure due to appression and coalescence of original haptere branches.

FIG. 6. Diagram of cross section through stipe 12<sup>cm</sup> above holdfast showing flat pith and zones of secondary thickening.

FIG. 7. Cross section through outer cortical area of stipe 1<sup>cm</sup> in diameter showing epidermis, cambial tissue, chlorenchyma, and transition to sclerenchyma.

FIG. 8. Cross section through zone of secondary thickening showing portion of a "ray" caused by the section striking the middle of adjacent cells in a radial row; between two of the cells button-shaped masses of cell-wall substance have been developed.

FIG. 9. Section through same area as last showing two "rays" cut at slightly different levels so that the shapes of the component cells are different; the majority of the cells have been cut across their arms giving a sclerenchymatous appearance.

FIG. 10. Cross section through the zone of tissue nearest to the pith showing the absence of arms and consequently of "rays," and illustrating the general sclerenchymatous appearance of this region.

FIG. 11. Cross section through the pith of same stipe showing the anastomosing web of hyphæ with cells packed with reserve materials.

FIG. 12. Longitudinal section through outer cortex and epidermis; transition from armed to outer cells.

## PLATE XXI.

FIG. 13. Longitudinal section through secondary growth zone of stipe illustrating the armed character of the cells and their arrangement side by side at the same level.

FIG. 14. Diagrammatic representation of the position maintained by the armed cells; cross sections at point *a* would show the ordinary sclerenchymatous structure, while those made at point *b* would show the "rays" as in *fig. 9*.

FIG. 15. Longitudinal section of stipe near the pith showing on the left hyphæ of the pith web, and on the right the sclerenchymatous cells of the inner growth zone.

FIG. 16. Diagram of cross and longitudinal sections through the petiole base of the lamina; *a*, outer cortex and chlorenchyma; *b*, parenchymatous tissue; *c*, sclerenchymatous layer around the pith; *d*, pith.

FIG. 17. Detail of cross section through same area as last, showing epidermal, chlorenchymatous, and parenchymatous tissue.

FIG. 18. Cross section same area as last and continuous with it, showing transition to perimedullary sclerenchyma with cells of the pith web running out from the central pith.

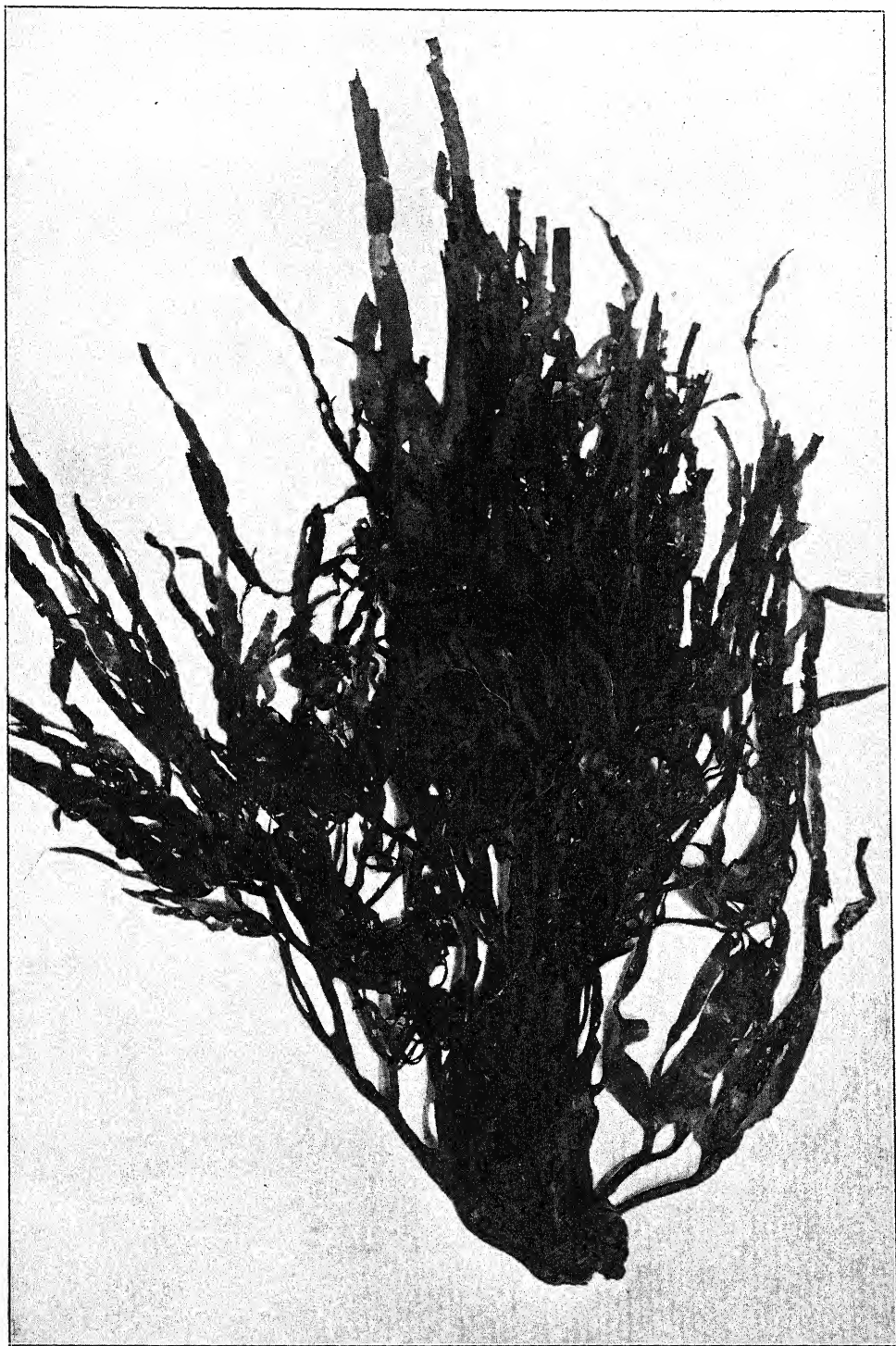
FIG. 19. Longitudinal section through the region of *fig. 17*.

FIG. 20. Longitudinal section through the region of *fig. 18*.

FIG. 21. Cross section of lamina through the wing.

FIG. 22. Section through a sorus showing sporangia with paraphyses, the latter capped by cuticular fragments corresponding to separate cells of the epidermis.

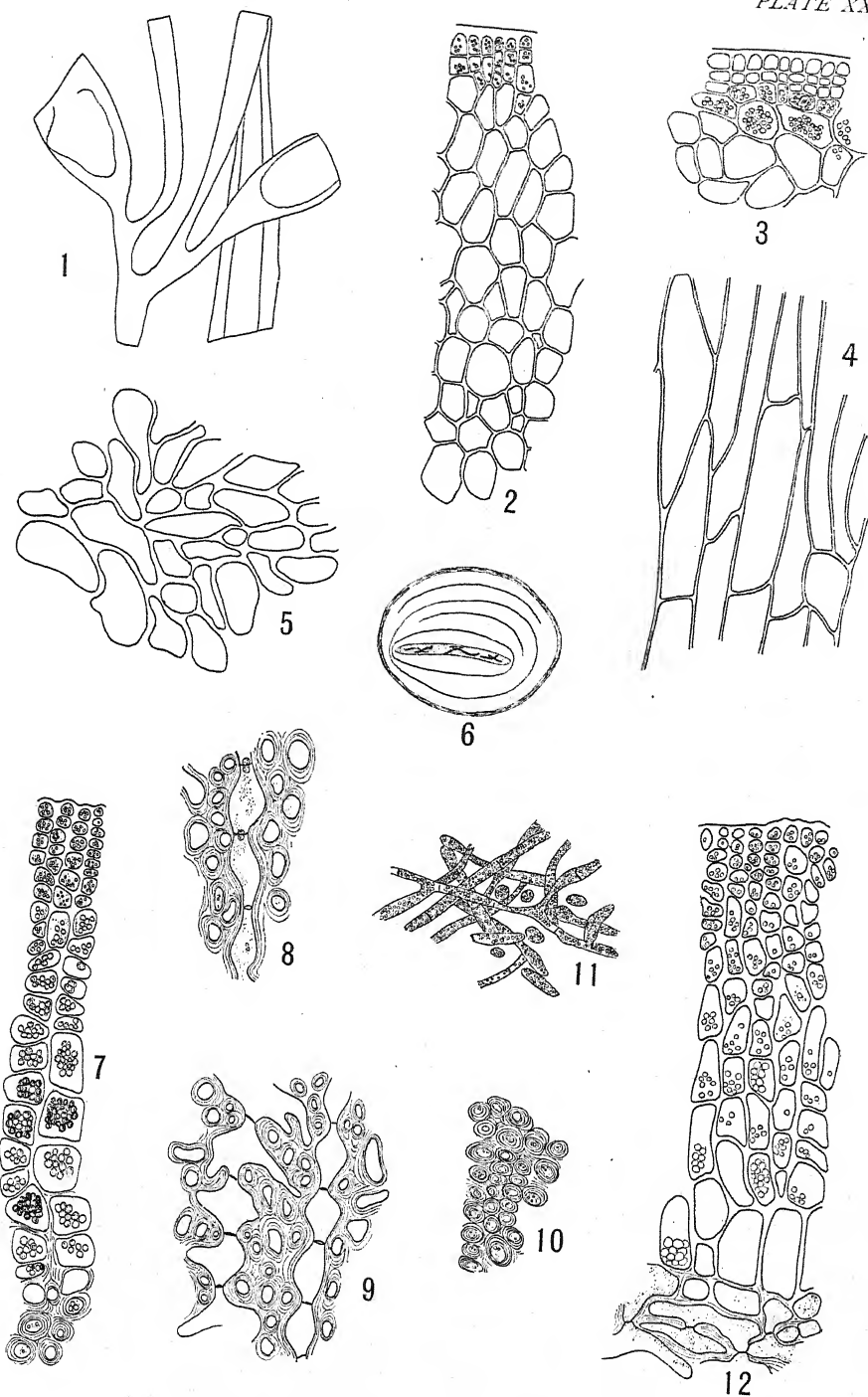




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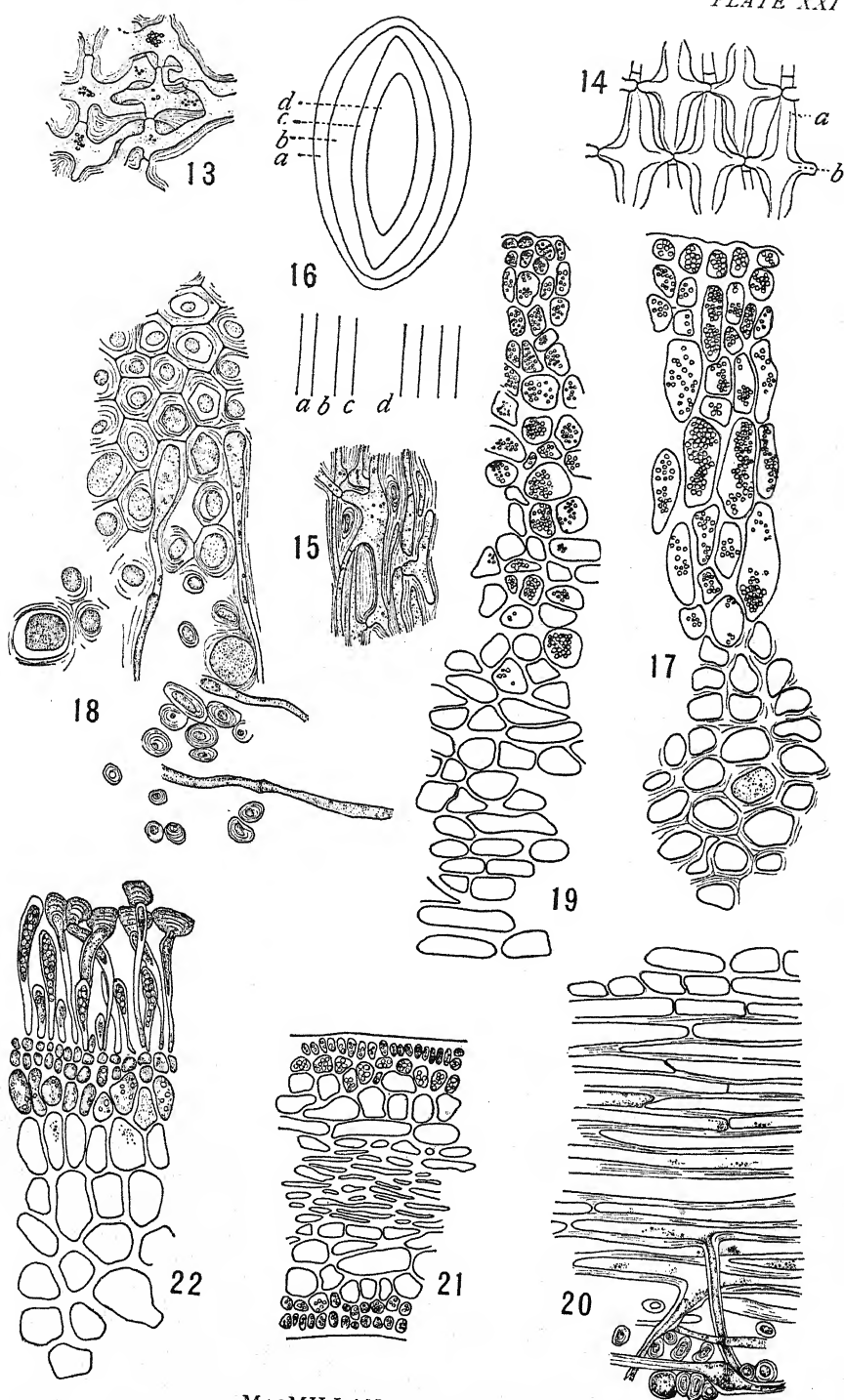






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## STUDIES IN CRATÆGUS. II.<sup>1</sup>

C. D. BEADLE.

*Cratægus aprica*, n. sp.—A large branching shrub, with one or several stems, 3–5<sup>m</sup> tall, or occasionally arborescent and attaining a height of 6–7<sup>m</sup> under favorable conditions: bark of the trunk and larger stems dark gray or nearly black, often conspicuously furrowed, the surface being broken into small, irregular, persistent, plate-like scales: branches ascending, armed with stout, either straight or slightly curved spines 2–6<sup>cm</sup> long, which are frequently branched and of greater size on the trunk and larger branches; branchlets at first villose-pubescent, but ultimately glabrous, marked by numerous small, pale lenticels, the bark reddish-brown, after the first winter changing to gray, with tinges of red or brown, the whole forming a compact, oval, or round head: winter buds globose, bright reddish-brown, the outer scales of the terminal ones thick and pointed: leaves thin to subcoriaceous in texture, obovate, rhombic-ovate, or orbicular in outline, 1.5–7<sup>cm</sup> long including the petiole, 1–5<sup>cm</sup> wide, the borders dentate or crenate-dentate and conspicuously glandular, more or less lobed near the acute apex, or on vigorous shoots more deeply lobed, especially below the middle of the blade, usually narrowed but sometimes rounded at the base and prolonged into a margined petiole 7<sup>mm</sup>–2<sup>cm</sup> long, which, like the base of the leaf, bears numerous black colored glands; stipules linear, linear-lanceolate, or on strong shoots foliaceous and lunate, pectinately-glandular or glandular-serrate, caducous: flowers, which appear in the vicinity of Biltmore, North Carolina (type locality), when the leaves are nearly grown, borne in 3–6-flowered, pubescent or pilose-pubescent, bracteate corymbs; pedicels 1–2<sup>cm</sup> long, pilose-pubescent, bearing one or two small pectinately-glandular, deciduous bractlets: calyx obconic, pubescent, at least near the

<sup>1</sup> Continued from BOT. GAZ. 28: 417. 1899.

base, the divisions 3-5<sup>mm</sup> long, glandular-serrate, or pectinately-glandular: petals rather broader than long, 9-13<sup>mm</sup> by 8-12<sup>mm</sup>, with a short broad claw at the base: stamens 10, 5-8<sup>mm</sup> long, the anthers light yellow: styles 3-5, surrounded at the base with pale hairs: fruit globose, 9-14<sup>mm</sup> in diameter, red or orange-red, ripening and falling after the middle of September, the flesh thick, yellowish, pleasant to the taste: nutlets 3-5, hard and bony, 6-8<sup>mm</sup> long, 3-4<sup>mm</sup> measured dorso-ventrally, the back ridged and grooved and the lateral faces nearly plane, a volume of 125<sup>cc</sup> containing about 1598 clean and dry seeds.

*Cratægus aprica* has been confounded with *C. flava* Ait.,<sup>2</sup> from which it differs in the shape and color of the fruit. The new species is abundantly represented in the mountainous region of North Carolina, and has been found in similar situations in Tennessee, Alabama, and Georgia, inhabiting sunny exposures in dry, rocky, or clayey soils.

The type material is preserved in the Biltmore herbarium.

*Cratægus sororia*, n. sp.—A tree 5-7<sup>m</sup> tall, with a trunk 1-1.5<sup>dm</sup> in diameter, dividing two or three meters above ground into several stout, ascending or spreading branches, which form an oval or round head; or usually smaller, 3-4<sup>m</sup> in height, forming a large shrub with one or more stems: bark gray, tinged with brown or nearly black, furrowed and broken on the surface into small, persistent scales: branchlets armed with gray or chestnut-brown spines 1.5-3.5<sup>cm</sup> long: buds globose, bright reddish-brown: leaves 2-6<sup>cm</sup> long, including the petiole, 1-3<sup>cm</sup> broad, or on vigorous shoots sometimes 6<sup>cm</sup> broad, obovate, round-ovate, or nearly orbicular in outline, or on the shoots even broader than long, with a truncate or subcordate base, acute or rounded at the apex, either gradually narrowed or abruptly contracted at the base and prolonged into a margined, glandular petiole 5<sup>mm</sup>-1.5<sup>cm</sup> long, the borders sharply and irregularly serrate and incisely lobed, especially above the middle, the serratures glandular-apiculate; sparingly pubescent when young (at least along the petiole, midrib, and principal veins), becoming glabrous; or with a few hairs in the axils of the prominent veins and bordering the

<sup>2</sup>Hort. Kew 2: 169. 1789.

petiole, bright green on the upper surface, paler below, fading in the autumn to tones of yellow and brown, or with occasional dashes of red: flowers, which appear in the vicinity of Rome, Georgia (type locality), during the last of April or first of May, and when the leaves are nearly grown, borne in pubescent, glandular-bracteate 3-6-flowered corymbs; pedicels 5-15<sup>mm</sup> long, sparsely pubescent, bearing one or more pectinately-glandular, caducous bractlets: calyx obconic, usually with a few soft hairs, the divisions 6-8<sup>mm</sup> long, glandular-serrate: stamens normally 20: styles 2-5, commonly 3, surrounded at the base with pale hairs: fruit large, globose, 12-18<sup>mm</sup> in diameter, red, red and yellow, or yellowish-red, ripening and falling after the middle of September, the flesh thick, soft, and pleasant to the taste: nutlets usually 3, hard and bony, 7-9<sup>mm</sup> long, 4-5<sup>mm</sup> thick, measured from the back to the inner angle, the lateral faces nearly plane and the back ridged and grooved.

*Cratægus sororia* is related to *C. aprica* above proposed and to *C. flava* Aiton, *l. c.* From the former it may be separated by the more numerous stamens, larger fruit and calyx segments, and coarser seeds; while from the last named species it differs from the accepted figures and descriptions which have been drawn from specimens in cultivation in Europe, in the shape of the fruit and the pubescent corymbs and petioles. The proposed species is abundantly represented on wooded hills, slopes, and rocky exposures, and in old fields from northwestern Georgia and adjacent Alabama southward to Florida.

The type material is preserved in the Biltmore Herbarium.

**Cratægus Alleghaniensis**, n. sp.—A large shrub 2-4<sup>m</sup> tall, or infrequently a small tree 5<sup>m</sup> in height: bark gray, sometimes tinged with brown or much blackened: branches ascending, armed with slender, gray or reddish-brown spines 1.5-4<sup>cm</sup> long, the whole forming an oval or round head; the growth of the season clothed with reddish-brown bark and marked by small, pale lenticels: leaves ovate, oval, or nearly orbicular in outline, 2-8<sup>cm</sup> long, including the petiole, 1.5-6<sup>cm</sup> wide, very sharply and irregularly serrate and incisely lobed, acute at the apex, either abruptly narrowed or rounded at the base, or on vigorous shoots subcordate and prolonged into a margined, glandular petiole

5<sup>mm</sup>–2<sup>cm</sup> long, bright green on the upper surface, slightly paler below and with 3–4 prominent pairs of veins, sparsely pubescent when young, especially on the upper surface, soon becoming glabrous: flowers, which open in the vicinity of Valley Head, Alabama (type locality), the first of May, disposed in simple, 3–6-flowered corymbs; pedicels 1–2<sup>cm</sup> long, bearing one or more small, linear, or lanceolate, pectinately-glandular caducous bractlets: calyx obconic, glabrous, the divisions 4–6<sup>mm</sup> long, glandular-serrate: petals 7–9<sup>mm</sup> broad, 9–12<sup>mm</sup> long, the claw at the base narrow: stamens normally 10, 4–6<sup>mm</sup> long, the anthers purple: styles 2–5, mostly 3–4, surrounded at the base with pale hairs: fruit, which ripens after the middle of September, globular-pyriform, red, 9–14<sup>mm</sup> long, 8–12<sup>mm</sup> broad: nutlets 2–5, usually 3–4, hard and bony, 5–7<sup>mm</sup> long, about 3<sup>mm</sup> thick measured from the back to inner angle, the lateral faces nearly plane and the back grooved and ridged.

*Cratægus Alleghaniensis* is abundant on Lookout mountain above Valley Head, Alabama, growing on rocky exposures or in the shade of oaks and pines. It is related to *C. aprica* above proposed, from which it may be distinguished by the less glandular foliage and inflorescence, the form and darker color of the fruit, purple anthers, and the sharply serrate borders of the leaf-blades.

The type material is preserved in the Biltmore Herbarium.

*Cratægus venusta* n. sp.—A tree seldom more than 8<sup>m</sup> tall, or frequently a large branching shrub growing on rocky slopes or occasionally on the banks of small streams: flowers, which open about April 20, in the vicinity of Birmingham, Alabama (type locality), and when the leaves are half grown, 2–2.5<sup>cm</sup> or occasionally more in diameter, disposed in 3–6-flowered corymbs; pedicels 1.5–2<sup>cm</sup> long, glabrous, bearing two or three pectinately-glandular, deciduous bractlets which vary from 5<sup>mm</sup> to 1.5<sup>cm</sup> in length and from 2–4<sup>mm</sup> in width: calyx obconic, smooth, the segments acute, 4–6<sup>mm</sup> long, 1.5–3<sup>mm</sup> wide, glandular-serrate or often pectinately-glandular below the middle: petals nearly orbicular, rather broader than long, 9–12<sup>mm</sup> wide, 8–10<sup>mm</sup> long, with a short and broad claw at the base: stamens normally 20, 4–8<sup>mm</sup> long, the anthers yellow: pistils 3–5, surrounded at the



base with pale hairs: fruit which ripens and falls after the first of October, globose or slightly oval, 9–13<sup>mm</sup> wide, 9–15<sup>mm</sup> long, dull red to greenish-red when fully ripe, sometimes, when more exposed, brighter, and frequently presenting surfaces of russet-red; the cavity 3–5<sup>mm</sup> broad and nearly as deep, surrounded by the remnants of the stamens: nutlets 3–5, hard and bony, 6–9<sup>mm</sup> long, 3.5–5<sup>mm</sup> measured dorso-ventrally with the lateral faces nearly plane and the back grooved and ridged: leaves thin to subcoriaceous, sparsely pubescent when young, soon smooth, bright green on the upper surface, paler below, showing 4–7 pairs of prominent veins, from obovate to ovate in outline, occasionally on strong shoots round-ovate, 2.5–12<sup>cm</sup> long, including the petiole, 1–6<sup>cm</sup> wide, acute at the apex, rounded or narrowed at the base into a narrowly winged and remotely glandular petiole 7<sup>mm</sup>–4<sup>cm</sup> long; the borders irregularly or doubly serrate and incisely lobed, with minutely glandular-tipped serratures: stipules linear or linear-lanceolate, pectinately-glandular, caducous: bark of the trunk varying from ashy-gray to light-brown, slightly fissured: branches spreading or ascending, bearing numerous stout, dark chestnut-brown, or gray spines 3–7<sup>cm</sup> long, or the older branches and frequently the trunk armed with strong much branched spines of greater size; the bark reddish-brown, marked by small, pale lenticels: buds globular, bright reddish-brown.

*Cratægus venusta* is abundantly represented in the Red Mountain region of Alabama, where it was apparently first discovered by Mr. C. L. Boynton of the Biltmore Herbarium, and later by Professor C. S. Sargent of the Arnold Arboretum. The new species is closely related to *C. Sargenti* Beadle,<sup>3</sup> differing mainly in the more numerous-flowered corymbs, shorter and stouter pedicels, yellow anthers, narrower and less persistent calyx lobes, and the strikingly obovate and elongated outline of the larger leaves.

The type material is preserved in the Biltmore Herbarium.

*Cratægus Ashei*, n. sp.—A tree seldom attaining a height of more than 6<sup>m</sup> or commonly a large branching shrub with one or more stems: bark of the trunk and older branches light gray or reddish-brown, smooth on the smaller plants, fissured and

<sup>3</sup> BOT. GAZ. 28: 407. 1899.

slightly scaly on the trunk of larger individuals: branches ascending, armed with stout, simple or branched, gray or chestnut-brown spines 2–5<sup>cm</sup> long, forming a pyramidal or oval head; branchlets at first pubescent, becoming smooth, the bark reddish-brown or gray, sprinkled with small, pale lenticels: buds globular, bright reddish-brown, the terminal on strong shoots with thick, acute, slightly spreading scales: leaves, which are about half grown at flowering time, ovate, round-ovate, or occasionally obovate, 3–9<sup>cm</sup> long including the petiole, 2–6<sup>cm</sup> wide, or occasionally larger on vigorous shoots, rounded or acute at the apex, abruptly contracted or wedge-shaped at the base and prolonged into a margined, pubescent petiole 5<sup>mm</sup>–2<sup>cm</sup> long, which, as well as the base of the blade, bears several sessile or stalked dark colored glands, the borders sharply and often doubly serrate, the serratures tipped with minute black glands, the upper surface pubescent, becoming nearly smooth with age, bright green and lustrous, pale green on the lower surface, more densely and permanently pubescent, especially along the midrib and principal veins, which are displayed in 5–7 pairs, texture firm to nearly coriaceous; stipules narrowly lanceolate, straight or falcate, frequently foliaceous and lunate on vigorous shoots, pectinately-glandular or glandular-serrate: flowers, which expand in the vicinity of Montgomery, Alabama (type locality), early in May, produced in simple or branched 3–10-flowered, glandular-bracteate, pubescent corymbs, 1.5–2.5<sup>cm</sup> in diameter: calyx obconic, pilose or pubescent, the divisions lanceolate, 7–10<sup>mm</sup> long, 3–5<sup>mm</sup> wide, smooth or nearly so on the outer surface, pubescent on the inner, pectinately-glandular or incisely glandular-serrate, reflexed after anthesis: petals slightly broader than long, 10–14<sup>mm</sup> by 9–12<sup>mm</sup>: stamens normally 20, 6–9<sup>mm</sup> long, the anthers yellow: styles 3–5, surrounded at the base with pale hairs: pedicels 1.5–3<sup>cm</sup> long, pubescent or pilose, bearing a linear or lanceolate, pectinately-glandular bractlet: fruit, which ripens and falls the last of September and early in October, red, more or less pubescent, globose, 10–14<sup>mm</sup> in diameter or occasionally slightly oval; cavity 4–6<sup>mm</sup> wide, surrounded

by the persistent calyx lobes and remnants of the stamens: nutlets 3-5, hard and bony, displaying a prominent ridge on the back, or conspicuously grooved and ridged, 6-9<sup>mm</sup> long, 4-6<sup>mm</sup> measured from the back to inner angle, the lateral faces nearly plane.

*Cratægus Ashei* has been found in the abandoned fields and woodlands, generally in clayey soil, of Montgomery county, Alabama. The species is related to *C. Harbisoni* Beadle,<sup>4</sup> from which it may be distinguished by the comparatively simple and less floriferous corymbs, more lucid and less pubescent foliage, and by the attenuated calyx lobes. I take pleasure in associating with this species the name of Mr. William Willard Ashe, forester of the geological survey of North Carolina.

The type material is preserved in the Biltmore Herbarium.

*Cratægus senta*, n. sp.—A small tree 5-6<sup>m</sup> high, or more frequently a large shrub with one or more stems, bark of the trunk rough, dark gray, usually much blackened near the base: branches spreading, slightly pendulous or recurved, zig-zag, clothed with smooth, dark or brownish-gray bark and armed with stout, gray or chestnut-brown spines 1.5-6<sup>cm</sup> long: leaves obovate, obovate-cuneiform, or on vigorous shoots round-ovate to nearly orbicular, 2-7<sup>cm</sup> long including the petiole, 7<sup>mm</sup>-5<sup>cm</sup> wide, prolonged into a margined, glandular petiole 5<sup>mm</sup>-2<sup>cm</sup> long, the borders deeply and irregularly serrate and incisely lobed, especially above the middle of the blade, the serratures glandular-tipped, bright green and shining on the upper surface, pale below and displaying several pairs of conspicuous ascending veins, pubescent at the time of unfolding, soon becoming nearly glabrous, except on the petioles and in the axils of the prominent veins, and fading to tones of yellow, red and brown: flowers, which appear early in May in the vicinity of Biltmore, North Carolina (type locality), and when the leaves are nearly grown, borne in simple, pubescent 3-6-flowered corymbs; pedicels 1-2.5<sup>cm</sup> long, densely pubescent when young, bearing one or two linear, caducous bractlets: calyx obconic, pubescent, the divisions 4-6<sup>mm</sup> long, glandular-serrate: petals 8-12<sup>mm</sup> in diameter, rather longer than broad, the claw at the base short and

<sup>4</sup> BOT. GAZ. 28: 413. 1899.

broad: stamens normally 20, 5-7<sup>mm</sup> long: styles 3-5, surrounded at the base with pale hairs: fruit globose, 10-14<sup>mm</sup> in diameter, red, ripening and falling the last of September and beginning of October: nutlets 3-5, hard and bony, 7-9<sup>mm</sup> long, 4-5<sup>mm</sup> measured from back to inner angle, the back shallowly grooved and the lateral faces nearly plane.

*Cratægus senta* is abundantly represented in abandoned fields and in open woods near Biltmore, North Carolina, and was referred to by me in an article published in the BOTANICAL GAZETTE<sup>5</sup> under the name *C. elliptica*. The new species is related to *C. Michauxi* Pers.<sup>6</sup> (*C. glandulosa* Michx.,<sup>7</sup> not Aiton or Willdenow), from which it may be recognized by the longer petioles and pedicels, the sharply cut and nearly smooth leaves and less glandular characters of foliage and inflorescence.

The type material is preserved in the Biltmore Herbarium.

*Cratægus Alabamensis*, n. sp.—A tree 4-6<sup>m</sup> tall, or more commonly a large branching shrub with one or more stems, and spreading, often pendulous branches: bark rough, gray tinged with brown, or much blackened near the base: branchlets at first villose-pubescent, often puberulous in their first winter, but ultimately glabrous, gray tinged with reddish-brown, or even brighter on the younger wood, slender, zig-zag, armed with dark gray or chestnut-brown spines 1.5-4<sup>cm</sup> long, or sometimes longer and branched on the older branches and trunk: winter buds globose, bright reddish-brown: leaves obovate or obovate-cuneiform, rounded at the apex and often with a short point at the end of the midrib or occasionally very abruptly contracted into an acute tip, gradually narrowed or cuneiform at the base and prolonged into a margined, pubescent, glandular petiole 7<sup>mm</sup>-2.5<sup>cm</sup> long, 2-7<sup>cm</sup> long including the petiole, 1-3.5<sup>cm</sup> wide, or even larger on the shoots, pubescent at the time of unfolding, especially along the principal veins, and at maturity subcoriaceous or thinner, bright green and lustrous above and pale on the lower surface, the borders crenate-dentate or serrate, especially above the middle, glandular-serrate or entire near the base; stipules linear or linear-oblong, on the strong shoots

<sup>5</sup> Bot. Gaz. 25: 447. 1898.

<sup>6</sup> Syn. Plant. 2: 38. 1807.

<sup>7</sup> Flora Bor.-Am. 1: 288. 1803.

lunate or variously lobed, pectinately-glandular or glandular-serate, caducous: flowers, which appear when the leaves are almost fully grown, borne in simple or branched, 3-9-flowered, densely-pubescent corymbs, and open in the vicinity of Montgomery, Alabama, (type locality), early in April; pedicels 1-2.5<sup>cm</sup> long, densely pubescent, bearing one or more small, glandular, caducous bractlets: calyx obconic, pubescent, the divisions 6-8<sup>mm</sup> long, glandular-serrate, reflexed after anthesis: petals orbicular or longer than broad, about 1<sup>cm</sup> in diameter, with a short and broad claw at the base: stamens normally 20, 5-7<sup>mm</sup> long, the anthers yellow: styles 2-5, usually 3, surrounded at the base with pale hairs: fruit large, elongated, 1.5-2<sup>cm</sup> long, 1-1.5<sup>cm</sup> wide, red, ripening early in August: nutlets usually 2-3 hard and bony, 8-10<sup>mm</sup> long, 3-4<sup>mm</sup> measured from the back to the inner angle, the lateral faces nearly plane and the back grooved and ridged.

*Cratægus Alabamensis* is abundant in dry, clayey soil near Montgomery, Alabama, growing in open woods or in cleared grounds. It is evidently related to *C. Michauxi* Pers. *l. c.*, from which it differs in form and size of the fruit, less glandular foliage, and attenuated calyx segments.

The type material is preserved in the Biltmore Herbarium.

*Cratægus pinetorum*, n. sp.—A shrub 1-5<sup>m</sup> tall growing in dry or rocky woods where the prevailing forest growth consists of pines, oaks, and hickories: stems one or more, clothed with smooth or roughened, dark gray bark, which is frequently blackened near the base: branches slender, armed with slender, straight or curved, dark gray or chestnut-brown spines 1-5<sup>cm</sup> long, the growth of the season covered with smooth, reddish-brown bark which is marked by small pale lenticels, becoming in the second year dark gray tinged with brown: leaves ovate, oval or obovate, 2.5-9<sup>cm</sup> long including the petiole, 1.5-5<sup>cm</sup> wide, sparingly pubescent on the midrib and veins on the upper surface when young, soon glabrous, acute at the apex, sharply and irregularly serrate and incisely lobed, the serratures minutely glandular-apiculate, narrowed or rounded at the base and prolonged into a margined, sparsely-glandular petiole 1-2.5<sup>cm</sup> long,

thin in texture, bright green on the upper surface, below paler and displaying 3-5 pairs of ascending, prominent veins; stipules linear or linear lanceolate, glandular, caducous: flowers, which open in the vicinity of Albertville, Alabama (type locality), about the first of May, and when the leaves are nearly grown; produced in simple, glandular-bracteate 3-6-flowered corymbs; pedicels 1-2<sup>cm</sup> long, bearing one or more narrow, pectinately-glandular, caducous bractlets; calyx obconic glabrous, the divisions 3-5<sup>mm</sup> long, glandular-serrate: petals orbicular, or a little broader than long, about 8-10<sup>mm</sup> in diameter: stamens normally 20, 4-6<sup>mm</sup> long: styles 3-5, surrounded at the base with pale hairs: fruit, which ripens in September, subglobose, 7-10<sup>mm</sup> in diameter, changing from tones of green and yellow to light red when fully ripe, the flesh thin: nutlets 3-5, hard and bony, 5-7<sup>mm</sup> long, 3-4<sup>mm</sup> measured from the back to the inner angle, the lateral faces nearly plane and the back ridged and grooved.

*Cratægus pinetorum* is probably related to and easily contrasted with *C. Boyntoni* Beadle<sup>8</sup>, from which it may be distinguished by the smaller fruit and more numerous stamens.

The type material is preserved in the Biltmore Herbarium.

*Cratægus rubella*, n. sp.—A shrub 1-4<sup>m</sup> tall, growing in upland woods: stems one or several, much branched, clothed with gray or reddish-brown bark, either smooth or slightly fissured and scaly: branchlets numerous, armed with slender, straight or slightly curved gray or chestnut-brown spines 1.5-4<sup>cm</sup> long, or on the older plants nearly destitute of spines back to the larger branches: leaves oval, ovate or obovate, 3-9<sup>cm</sup> long including the petiole, 1.5-4.5<sup>cm</sup> broad, thin at first, becoming firm in texture, sparingly pubescent when young especially on the upper surface, soon glabrous, sharply and doubly serrate to near the base, and incisely lobed above the middle of the blade, acute at the apex, narrowed at the base and prolonged into a margined, sparsely-glandular petiole 1-2.5<sup>cm</sup> long, reddish-green or purplish at the time of unfolding, becoming bright green above, paler below and fading with decided tones of yellow; stipules oblong

<sup>8</sup> Bot. Gaz. 28: 409. 1899.

or linear-lanceolate, pectinately-glandular, early deciduous: flowers, which appear when the leaves are nearly grown, borne in simple, 3-6-flowered glandular-bracteate corymbs, and opening in the vicinity of Valley Head, Alabama (type locality), about the first of May; pedicels 1-2<sup>cm</sup> long, bearing one to three pectinately-glandular, deciduous bractlets: calyx obconic, the segments 4-6<sup>mm</sup> long, glandular-serrate: petals rather broader than long, 8-12<sup>mm</sup> wide, 7-10<sup>mm</sup> long, with a short, broad claw at the base: stamens normally 10, sometimes united in pairs and appearing to be fewer, 5-7<sup>mm</sup> long, the anthers light purple: styles 2-4, rarely 5, surrounded at the base with pale hairs: fruit red, pyriform or oval, 12-15<sup>mm</sup> long, 10-12<sup>mm</sup> wide, ripening after the middle of September: nutlets 2-3, rarely 4-5, hard and bony, 6-7<sup>mm</sup> long, 3-4<sup>mm</sup> measured dorso-ventrally, the lateral faces nearly plane and the back ridged and grooved.

*Cratægus rubella* is abundant on Lookout mountain above Valley Head, Alabama, growing in the shade of oaks and pines, and has been collected in similar situations in eastern Tennessee and western North Carolina. It has been customary to refer this species to *C. flava*, *C. rotundifolia*, and *C. coccinea*, but I am inclined to place it near and compare it with *C. Boyntoni* Beadle, *l. c.*, from which it differs conspicuously in the outline of the leaves, shape and color of the fruit, and the purple color of the anthers.

The type material is preserved in the Biltmore Herbarium.

*Cratægus straminea*, n. sp.—A low shrub, about 1<sup>m</sup> in height, frequently growing in large patches in upland woods, or occasionally attaining larger proportions, 2-3<sup>m</sup> tall, and developing from one or more stems a coarse shrub with loose or straggling outline: branches gray, tinged with brown or reddish-brown, armed with slender, curved or straight spines 1.5-6<sup>cm</sup> long which vary from gray to chestnut-brown in color: leaves oval, ovate or round-ovate in outline, acute at the apex, abruptly contracted or rounded, or on vigorous shoots truncate or subcordate at the base, acutely incised or slightly 5-9 lobed, and sharply and irregularly serrate except at the extreme base of the blade, the serratures glandular-apiculate, thin to subcoriaceous in texture, 2.5-10<sup>cm</sup> long including the petiole, 1.5-6<sup>cm</sup> broad, sparingly

pubescent when young, becoming glabrous with age, or with a few hairs along the midrib and principal veins which are disposed in 3-5 pairs; petioles winged, 7<sup>mm</sup>-3.5<sup>cm</sup> long, bearing several or many stalked glands; stipules linear or linear-oblong, pectinately-glandular, caducous: flowers, which appear in the vicinity of Valley Head, Alabama (type locality), and when the leaves are nearly grown, disposed in glandular-bracteate, 3-6-flowered corymbs; pedicels 7<sup>mm</sup>-2.5<sup>cm</sup> long, bearing one or two, pectinately-glandular, caducous bractlets; calyx obconic, the divisions 4-6<sup>mm</sup> long, glandular-serrate and with a few stalked glands below the middle: petals nearly orbicular, 6-10<sup>mm</sup> in diameter, with a short, broad claw at the base: stamens normally 10, 5-7<sup>mm</sup> long, the anthers purplish: styles 3-5, surrounded at the base with pale hairs: fruit subglobose or pyriform, 10-13<sup>mm</sup> high, 9-11<sup>mm</sup> wide, yellow or greenish-yellow, ripening after the middle of September: nutlets 3-5, hard and bony, 7-8<sup>mm</sup> long, 3-4<sup>mm</sup> measured from the back to the inner angle, the lateral faces nearly plane and the back grooved and ridged.

*Crataegus straminea* frequently covers large areas on the top of Lookout mountain above Valley Head, Alabama, growing in the shade of oak, pine, and hickory trees, and will probably be found to extend into the adjacent regions of Tennessee and Georgia. It is related to *C. Boyntoni* Beadle, *l. c.*, from which it differs in habit of growth, form and color of fruit, color of the anthers, slender spines, and leaves with more sharply cut borders.

The type material is preserved in the Biltmore Herbarium.

BILTMORE HERBARIUM,  
Biltmore, N. C.



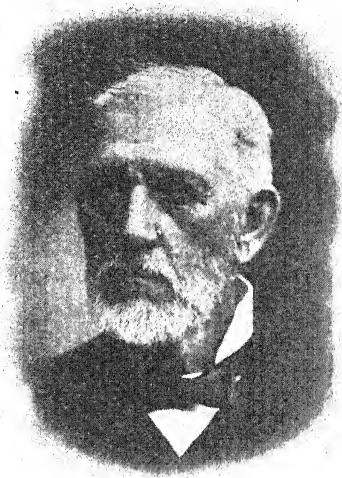
## BRIEFER ARTICLES.

DAVID FISHER DAY

(WITH PORTRAIT)

JUDGE DAVID F. DAY, who died on August 21, 1900, was born in Buffalo, N. Y., in 1829, and his whole life was spent in that city. For more than fifty years he was engaged in the practice of the law, in which profession he held high rank.

His love of nature, which was that of an enthusiast, early led him to the study of the natural sciences, in which he became most proficient. As a field botanist he was excelled by few. Gifted with a remarkable memory and a particularly clear conception of the relationships of families and genera, he was able to place a new plant with a facility that I have seldom seen equaled. His methods of reasoning upon botanical problems were not always the methods of the schools, and his way of approaching a question from an unusual standpoint was refreshing and often the means of solving difficulties of long standing. The fact that he was not a professional



botanist seems to have deterred him from publishing many of his observations and deductions, which were of great interest and value.

In connection with Judge Clinton he made a thorough study of the local flora, and the result appeared in "A catalogue of the plants of Buffalo and vicinity," published in 1883. Later he prepared a list of plants growing on the reservation at Niagara Falls, which was published by the state. He was one of the founders of the Buffalo Society

of Natural Sciences, and a life-long member of it. To him also is due the credit of establishing the Buffalo Botanic Garden, in which he was particularly interested.

His library was a notable one, as he was a collector of rare discrimination, and the works on botany were many and valuable. In addition to the more pretentious volumes, a large collection of pamphlets was accumulated, among which appear a very large number of local catalogues. These books of botanical interest, as well as his collections of living plants and herbarium specimens, he was preparing to transfer to the Botanic Garden when he was stricken down.

Mr. Day had for years been a member of the Park Commission of his city, and drew the act which created the Park department. In this act, drawn thirty years ago, he made provision for both botanical and zoological collections, both of which, after years of waiting, he saw established.

His loss will be mourned by the many botanists of his acquaintance, as well as by his fellow-citizens, by whom he was held in high esteem. —JOHN F. COWELL, *Buffalo Botanic Garden*.

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#### OBSERVATIONS ON THE ROOT SYSTEM OF CERTAIN CACTACEÆ.

THOSE who make botanical trips into arid regions do so generally with the one idea of collecting material to be worked up at leisure in laboratories or herbaria, or to be deposited in botanic gardens. Their stay, as a rule, is closely limited, and the necessity of covering a great amount of ground brings with it a tendency to pass over those details which take considerable time. The root system of desert plants, both as regards structure and distribution of roots, is one of those questions which has perforce been to a great extent neglected, our knowledge, generally speaking, being confined to the examination of the amount of underground growth collected with herbarium specimens. Therefore it seemed to me worth while during my stay in Tucson, Arizona, in the midst of the great cactus plains, to make a rather careful study of the roots of certain large forms which could scarcely be preserved *in toto*.

The root systems of Cactaceæ, in general, are somewhat smaller than would be expected. The distribution, however, is such as in a way to make good the deficiency in size and length. The specific

examples here cited may be fairly taken, I think, as types, as without exception they agree in general characters.

*Echinocactus Wislizeni* Engelm.—Plants about 7.5<sup>dm</sup> high and 3.5 to 4<sup>dm</sup> in diameter gave the following results. Three or four main roots, 10 to 12<sup>mm</sup> thick, branch out horizontally from beneath the plant, taper but slightly, are sinuous and much branched at the tips, in such a manner as pretty thoroughly to cover the ground about the plant within a radius of 2.4<sup>m</sup>. The fine rootlets are very numerous, generally turning upward. In no place does this set of roots run much more than 10<sup>cm</sup> below the surface. Just beneath the plant are a few small roots passing almost directly downward, tapering very rapidly, and with numerous branchings penetrating to the depth of scarcely 30<sup>cm</sup>.

*Opuntia fulgida* Engelm.—An arborescent form. A specimen about 15<sup>dm</sup> high, considerably branched, showed, as in the above case, several long slender roots with fine branches, running horizontally 5 to 10<sup>cm</sup> below the surface, and covering an area with a radius of 30<sup>dm</sup> about the plant. In a plant of *O. fulgida mamillata* (Schott) Coulter, which was washed by a small torrent, these roots appeared above ground in many places. In both of these specimens a few quickly tapering roots were demonstrated at the base of the plant, passing directly downward to the depth of about a foot.

*Opuntia Whipplei* Engelm. showed a similar state of affairs.

A *Platopuntia* (*O. angustata* Engelm.?) possessed much the same characters. The prostrate younger joints here send out roots and reproduce by innovation,<sup>1</sup> but the chief roots are to be found attached to the oldest living joint. At this point a double system can be seen, consisting of a couple of large roots, horizontal and but slightly below the surface, and one or two much smaller roots passing downward with rapid branching.

*Cereus giganteus* Engelm.—This plant thrives on rocky hillsides, and its root system is modified somewhat by conditions of soil and slope. In the specimen examined the roots which passed directly downward came very quickly in contact with a flaky bedrock, into the crevices of which they sent their branches, sometimes causing a splitting off of fragments. Although these roots seemed to pass scarcely three feet downward, their hold was exceedingly firm. The horizontal roots were directed almost entirely up the slope, sometimes appearing on the surface, but often descending to some little depth to avoid large

<sup>1</sup> TOUMEY, J. W.: Vegetal dissemination in *Opuntia*. BOT. GAZ. 20: 356. 1895.

bowlders. The area which they covered extended about 45<sup>dm</sup> away from the plant, which was between 12 and 15<sup>m</sup> high.

*Cereus enneacanthus* Engelm.—This rather ascending, cespitose form showed, as in the other cases, the two sets of roots; horizontal, long and sinuous; and vertical, abruptly tapering and much-branched. Several specimens were examined.

CONCLUSIONS.—In the majority of the larger Cactaceæ there are two distinct root systems—one horizontal, for absorptive purposes; the other passing downward, for anchorage. The depth of the horizontal system varies with the degree of penetration of surface water, which, in turn, is to some extent dependent on the character of the soil. One of the specimens of *Echinocactus* was examined the day following a gentle rain which had continued intermittently for two days, thus giving the water time to soak in as it fell. The soil was dampened to a distance of about four inches below the surface, just the depth of the absorptive root system.

Observations upon young plants of *Opuntia fulgida* give the following as the sequence of root formation. The first to be developed by joint or seedling are the vertical roots, which for a time act both for absorption and anchorage. As the plant increases in size the ground directly beneath it is shielded from moisture by its body, and in order to get the necessary food material the horizontal system is developed secondarily, starting, as a rule, directly from the stem, but occasionally appearing in part as horizontal branches of vertical roots. This horizontal system covers an area far greater than that sheltered by the parts above ground. As the soil is generally firm, and the horizontal roots give some support, there is little need for any roots to descend to a great depth. The small amount of surface exposed to winds may be considered another reason for this.

Correlated with the difference in distribution of the two systems is a difference in structure, as may be proved by a most superficial examination. The horizontal roots are in all cases extremely brittle, the vertical much more elastic and capable of withstanding far greater tension. The woody cylinder in large absorptive roots is far smaller relatively than that of the anchoring roots. Microscopically this difference may be most easily seen in the secondary xylem. For this examination sections of roots of *Opuntia fulgida* and *Echinocactus Wislizeni* were used. The xylem of the absorptive root is composed mainly of ducts, that of the anchoring roots, which, after the appearance of the

horizontal system, seem to have developed for this purpose entirely, consists almost wholly of wood cells. Owing probably to the general evenness of climate the annual rings are not easily demonstrated.—CARLETON E. PRESTON, *Harvard University*.

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#### NON-SEXUAL PROPAGATION OF OPUNTIA.

PROFESSOR TOUMEY, in an article in the BOTANICAL GAZETTE (20:356. 1895), speaks in general terms of the use of spines as aids in dissemination of opuntias which are dispersed by the breaking off of the separate joints. A short note may be added as to the function of these spines, especially in such long-spined species as *O. fulgida* Engelm. A joint falling upon the sand very often rebounds from the elasticity of the spines, and by this impetus is carried some distance from the parent plant. The greatest aid, however, is in the placing of the joint. Joints destined for such dissemination are as a rule obovate, the best developed areolae with the longest spines being situated on the distal end, those of the proximal end being scarcely at all armed. The result of this is that the joint upon falling almost invariably lights with its base downward, in the best possible position for striking root. The distal parts are kept off the ground in all cases by the long spines.—CARLETON E. PRESTON, *Harvard University*.

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#### GAURELLA=GAUROPSIS.

I HAVE to propose the restoration of the name of *Gauropsis* Torrey & Fremont (Rep. 315. 1845), to take the place of *Gaurella* Small (Bull. Torr. Bot. Club 23:183. 1896). *Gauropsis* was clearly defined by its authors, though not treated as a genus, and the type and only species was the *Enothera canescens* Torrey & Fremont, described at the place cited. The *Index Kewensis* gives *Gauropsis* Presl, 1849; I have not seen Presl's work, but in any event it is later than that of Torrey and Fremont. The type species of *Gauropsis*, *Gaurella guttulata* (Geyer) Small will become *Gauropsis guttulata*, or, I think, much better *Gauropsis canescens*, since the name *canescens* is only preoccupied by a slight variety of *Enothera biennis*.—T. D. A. COCKERELL, *East Las Vegas, N. M.*

## CURRENT LITERATURE.

### BOOK REVIEWS.

#### Fossil plants.

MORPHOLOGISTS have made but little use of the work of paleobotanists, both on account of the nature of the material and because paleobotanists too often have not been trained morphologists. The time for this feeling, however, has passed, and some of the most striking extensions of our morphological horizon have come from the work of paleobotanists. What was needed more than any thing else was the critical sifting of the vast accumulation of paleobotanical data from the standpoint of modern botany. In 1891, Count Solms-Laubach published his admirable *Fossil Botany*, which has ever since remained a standard work for botanists, although more destructive of previous claims than constructive. Very recently, however, Mr. A. C. Seward has begun his great work on *Fossil Plants*, but it has not yet included the vascular plants; Professor R. Potonié has published his *Lehrbuch der Pflanzenpaleontologie*, a very compact presentation of the subject; and M. R. Zeiller's *Éléments de Paléobotanique* has just appeared. All of these books stand for the newer paleobotany, which is to reject uncertain evidence and rest upon a foundation of morphology.

What the morphologist wishes, however, is the omission of details which are not pertinent to him, and the compact presentation of what paleobotany has definitely contributed to morphology. This has been done, and apparently well done, by Professor D. H. Scott, in a book which has just appeared.\* His purpose has been "to present to the botanical reader those results of paleontological inquiry which appear to be of fundamental importance from the botanist's point of view." Since the important results have related almost entirely to pteridophytes and gymnosperms, the *Studies* are restricted to them. It is certainly true that Professor Scott has presented in a remarkably clear way just what botanists wish to know, but his data must be judged by paleobotanists. The book must be read for details, but some of the most striking suggestions for the consideration of morphologists are as follows.

The remarkable elaboration of Equisetales and Lycopodiales in the Paleozoic is fully set forth, and should form a part of every morphological presentation of these groups, the living representatives being totally inadequate for such a purpose. The most suggestive conclusion in this connection, however, is that these two groups, which seem to be so far apart now,

\* SCOTT, DUNKINFIELD HENRY: *Studies in fossil botany*. 8vo. pp. xiii + 533, with 151 illustrations. London: Adam and Charles Black. 1900. 7s. 6d.

are but divergent lines from an ancient stock represented by the Sphenophyllales. Professor Scott regards the Sphenophyllales as worthy to stand as a gymnosperm group of primary rank. He would include in it not only Sphenophyllum and its acknowledged associates, but Cheirostrobos as well, a genus which strikingly combines the characters of Equisetales and Lycopodiales.

Of course the Filicales are fully considered, a group of enormous antiquity, having held its own since the Silurian, and whose habit was so predominant during the Paleozoic that many plants have been included with them whose real affinities are elsewhere. In general, Professor Bower's grouping of Filicales into the Simplices, Gradatae, and Mixtae is shown to have an historical basis.

One of the most interesting sections of the book is that devoted to a consideration of the Cycadofilices, a provisional Paleozoic group made to contain forms which combine the characters of Filicales and Cycadales, and most of them first described as ferns. The representative genera are *Lyginodendron*, *Heterangium*, and *Medullosa*, whose habits and anatomical features are strikingly intermediate, but whose spore-bearing members are unfortunately unknown. It is evident, however, that in them we have forms which strongly confirm the conclusion that cycads have been derived from ferns.

As was to be expected, the dominant gymnosperm group of the Paleozoic, Cordaitales, is set apart as of primary rank, and, associated with the Cycadofilices, seems to furnish the background for later gymnosperm development.

The author regards the Bennettitæ as but a very distinct phylum of the Cycadales, but to the reviewer it would appear that they are worthy of primary rank and the name Bennettitales. Their habit and anatomical structure seem to be almost identical with those of Cycadales, but the lateral sporangium-bearing shoots, the fern-like "ramenta," and more than all the remarkably modified and complex ovulate strobili and peculiar embryo, justify the claim for independence. This is further justified by the steadiness maintained by the peculiar structures throughout the numerous European forms, and in the still more extensive display of the group in the rich Mesozoic flora of the United States, from which Professor Ward has described numerous species under the generic name *Cycadoidea*. In this connection it is interesting to note the recent description by Dr. Wieland of the staminate strobili of *Cycadoidea ingens* Ward. The numerous sporophylls are borne upon a central axis, instead of upon a hemispherical receptacle as in the ovulate strobili, and each sporophyll bears a considerable number of linear sori, each sorus composed of twenty to forty clavate sporangia. It seems that the Mesozoic has obtained the name of the "age of cycads" from the display of Bennettitales, and that while doubtless true cycads existed, and among them the genus *Cycas*, they are somewhat indefinite and uncertain.

In discussing the general problem of the phylogeny of gymnosperms, the author fairly states the contending claims for its polyphyletic and monophyletic origin, but plainly inclines to the latter position. He sees no reason for imagining any connection whatsoever with the Lycopodiales, and still less with the Equisetales. The connection of Cycadales and Bennettiales with Filicales being considered as established, both through morphology and the paleobotanical evidence of such a group as Cycadofilices, "the derivation of the other gymnosperms centers about the extinct group Cordaitales," whose affinities with the Cycadales are clear.—J. M. C.

#### Tropical Nature.

COSTANTIN, the well-known French botanical philosopher, has issued a second volume in the series known as Bibliothèque Scientifique Internationale. His studies in various lines of botanical research, and especially his classic ecological papers on aquatic plants, have well fitted Costantin, both for his work on Adaptation-Evolution<sup>2</sup> and for the work which is the subject of this review.<sup>3</sup> In the introductory chapters the author gives a realistic picture of the virgin forest of the tropics, and vividly recounts the first sensations that one has in these new scenes. After a few remarks on tropical climates, Costantin plunges at once into the very deepest ecological problems of the tropics. He first discusses the origin of forests, and takes up some of the types of trees that are peculiar to tropical forests. An interesting chapter on leaves follows, and some of the most recent studies, as those on hydathodes, are introduced. After a few pages on flowers and fruits, the author gives a brief survey of the geological history of forests, holding to the older view that the present tropical forests are fragmentary, but yet lineal descendants of those which have passed away. The lianas come in for an excellent treatment, four chapters being devoted to a discussion of the various classes and their origin. Chapters follow on epiphytes, especially those found among the tree tops; parasites of various classes; symbiosis, one chapter dealing with saprophytes, the other with the rôle of ants and other animals in influencing plant life. Saprophytism at one time would not have been given as the type of symbiosis, but recent studies on mycorrhiza makes such a classification necessary. The strictly botanical part of the volume closes with a chapter on mangroves and one on island floras, the latter from the geographic standpoint.

Though the volume is entitled *Tropical Nature*, it is confined practically to the plant world of the tropics. The last chapter is a curious exception and

<sup>2</sup> Les végétaux et les milieux cosmiques (Adaptation-Evolution). 292 pp., with 171 figures in the text. Paris. 1898.

<sup>3</sup> COSTANTIN, J.: La nature tropicale. 8vo. pp. 315. figs. 166. Paris: Félix Alcan. 1899. 6 fr.



deals with religious legends and traditions of the human race. This seems out of keeping with the rest of the work, which, though planned for a semi-popular audience, contains much matter of equal value to ecologists with the works of Haberlandt, Schimper, and Warming. The figures are crude and unsatisfactory in the extreme, when compared with Schimper. Though little that is new is to be found in Costantin's new volume, one finds there one of the best summaries we have of many topics, such as lianas and parasites. In the author's philosophy, also, is a great deal of valuable suggestion to all who are interested in ecology.—HENRY C. COWLES.

#### Organography.

TO THE admirably selected series of German botanical works translated into English for the Clarendon Press, the directors have now added Goebel's *Organography of Plants*.<sup>4</sup> This book is the only recent one dealing with morphology in its modern revival under the influence of experimental physiology. We have already written at length of the first part of this work upon its appearance,<sup>5</sup> and nothing need be added now.

The translation is by Professor Isaac Bayley Balfour of the University of Edinburgh, and in the main is very satisfactory. Intensification or weakening of the author's meaning occurs here and there; an occasional archaism, like "the seldom occurrence," p. 68, has crept in; now and then one finds a badly constructed sentence, as "one would naturally expect that a lateral shoot removed from the shoot system and planted vertically and which rooted would behave similarly," p. 51; and we much regret that the incorrect form "Spermaphyta" should be given further currency by the translator. But these defects are such as may be charitably overlooked in view of the great advantage of having this classic in English dress. We trust that the publication of the second part (with its promised index) will be made possible at an early day by the appearance of the remainder of the second part from the author's hand. The imprint of the Clarendon Press is guarantee that the manufacture of the book is all that can be desired.—C. R. B.

#### NOTES FOR STUDENTS.

M. H. DEVAUX has recently made an extensive study of lenticels, especially as concerns the physiological conditions of the growth and differentiation of their tissues, the results of which occupy two thirds of a volume of the *Annales des Sciences Naturelles*.<sup>6</sup>

<sup>4</sup>GOEBEL, K.: *Organography of plants, especially of the Archegoniata and Spermaphyta*. Authorized English edition by ISAAC BAYLEY BALFOUR. Part I: General Organography. Roy. 8vo. pp. xvi + 270. figs. 130. Oxford: The Clarendon Press. 1900.

<sup>5</sup>BOT. GAZ. 25: 290. 1898.

<sup>6</sup>DEVAUX, H.: *Recherches sur les lenticelles; étude sur les conditions physiologiques de l'accroissement et de la différenciation de la cellule et des tissus*. Ann. d. Sci. Nat. Bot. VIII. 12: 1-240. pl. 6. 1900.

The memoir is too voluminous to permit an adequate summary in the space at command, and the original should be available in every laboratory where research is prosecuted. Devaux gives a résumé of his work in the final chapter, from which some extracts are here made.

Lenticels are found in all the great groups of vascular plants and on all their organs which have secondary growth. The primary number on the stem is in ratio with the vigor of growth (indicated by the length of shoots or internodes), with the total number of internodes, and with the numerical rank of each internode. The curve of their production agrees closely with the curve of elongation of the internode, though the maxima do not exactly coincide. In each internode the lenticels are almost always more numerous on the distal moiety. The dimensions attained by lenticels depends directly on their number per unit of surface.

Two types are distinguished: (1) the closing layers are few in number, consisting of cells intimately united, with no (or small) intercellular spaces, being oftentimes comparable to true cork; (2) the closing layers are numerous, composed of rounded cells with many large intercellular spaces, and are like the packing cells (*Füllzellen*; *cellules comblantes*), but suberized. The packing cells are alike in both.

As to origin, lenticels are primary or secondary. Primary lenticels are formed early and at points determined by an organ (stoma, rootlet, less often a bud). Secondary lenticels are formed later and at points not determined by an organ. Whenever stomata are present there is a tendency to produce lenticels below them, usually in the cortex, sometimes in the pericycle. If stomata are very numerous, partial or complete abortion of some lenticels may occur. Some stems, wanting stomata, produce lenticels late, in the neighborhood of a bud after the fall of the leaf. Lenticels are likewise generally produced at the base of young rootlets, though equally subject to abortion. Lenticels are continually growing and continually being destroyed. The growth is due to continuous proliferation of the cells, the destruction to death or suberization of the cells. For the most part, proliferation produces complete rupture of the closing layers, especially in the spring, followed by hypertrophy, death, or suberization (accompanied or not by sclerosis), and by centripetal displacement of the cambium or its transformation into permanent tissue, to be regenerated later at a deeper level.

The lenticels are usually porose, but sometimes a complete closure occurs, not in winter only but throughout the year. Thanks to this porosity they certainly serve in large measure in the general gaseous exchanges of the organs. But it would be false to say that the lenticels exist for these exchanges. For (1) they are often absent or insufficient; (2) often the plant has porose regions different from lenticels; (3) opening and closing of the lenticels is not due to the needs of aeration. Rather they are organs of transpiration and automatic regulators of the internal moisture, which the

plant uses efficiently for the proper gaseous exchanges also. (M. Devaux promises a further memoir on the general aeration of the plant by lenticels.)

Finally, lenticels are defined as small limited regions of the superficial parenchyma in continual proliferation and continual development, capable of hypertrophy or of cicatrization, according to the conditions of external or internal humidity.—C. R. B.

A RECENT PAPER<sup>7</sup> on the embryology of *Taxus baccata* fills in some of the gaps in previous accounts. The writer secured an abundance of wild material, but employed rather primitive methods in making his preparations, fixing in absolute alcohol, imbedding in celloidin, and staining in haematoxylin. The following is a brief résumé of his work.

The origin of the aril shows it to be a second integument. In tracing the development of the embryo sac the author was not able to get the earliest stages. The two, four, and eight-celled stages of the embryo sac were not observed, but many sacs were observed in later stages. Free nuclear division continues until there are about 256 free nuclei in the sac (the eighth division), when cell walls begin to appear. These cells rarely contain more than one nucleus, and have a regular six-sided appearance in optical section. In the later stages of endosperm formation the cells are often multinucleate. The formation of archegonia begins at the end of May or the first of June, but archegonia in very different stages of development are soon found in the same prothallium, even embryos and young archegonia often appearing together. The usual number of archegonia is from five to eight, but nine, ten, and even eleven were observed. Pollination occurs from the beginning to the middle of March, and there is no so-called pollen chamber. By the end of May three nuclei can be seen in the pollen tube, the tube nucleus, and the nuclei of the stalk cell and of the generative cell. Shortly before fertilization occurs the generative cell divides into two very unequal cells. Fertilization takes place about the first of June. There may be several pollen tubes, and several "archegonia" may be fertilized. The sex nuclei, which are of about the same size, come into contact and then sink to the bottom of the archegonium, where fusion takes place. Nuclear division then proceeds until there are from ten to sixteen free nuclei at the base of the archegonium, after which the free cells become arranged into tiers, the upper tier ("rosette"), the middle tier, consisting of suspensor cells usually six in number but sometimes more, and the lower tier, which is the embryo proper. In *Taxus baccata* all the suspensors of one archegonium belong to a single embryo. There are normally two cotyledons, but in one case three were observed.—CHARLES J. CHAMBERLAIN.

<sup>7</sup> JÄGER, L.: Beiträge zur Kenntniss der Endospermbildung und zur Embryologie von *Taxus baccata* L. 'Flora 85: 241-288. pls. 15-19. 1899.

KAHLENBERG AND AUSTIN have been continuing the earlier researches on the toxic action of various substances on seedlings. They conclude<sup>8</sup> that the toxicity of acid sodium salts is greater than it ought to be if it were due solely to H ions and that the theory of electrolytic dissociation is unsatisfactory in explaining the poisonous action of these acid salts and of acids as well. The true explanation, they suggest, is very likely to be found in the ability which the substances all have in common, to neutralize basic substances. This explanation is independent of the theory of electrolytic dissociation. Kahlenberg also found himself unable to explain the sour taste of acids on the basis of the H ions<sup>9</sup> and holds the physiological effect to be due to their chemical activity in virtue of the fact that the H is replaceable by a metal of a basic radical. The more readily the H is replaced, the more reactive the acids are and the more intense is their taste.—C. R. B.

DR. GINO POLLACCI published a year ago the results of some of his researches on photosynthesis<sup>10</sup> which have not been adequately noticed in this journal. His most important results are as follows:

Green organs of plants which grow in sunlight give the aldehyde reaction with Schiff's reagent. Under the same conditions fungi do not so react; nor do leaves, kept for some hours in darkness or in an atmosphere free of CO<sub>2</sub>. Formic aldehyde reactions are also obtained from expressed sap by proper treatment. He holds, therefore, that formic aldehyde is produced by green organs under the normal conditions of photosynthesis, and promises to give the results of his researches on the process of its formation in a second memoir—C. R. B.

THE CORRECTION of a large number of typographical and other errors, both of omission and commission, in Engler and Prantl's *Pflanzenfamilien*, especially in the general index, will be found in *Allgemeine Bot. Zeitschrift* 1900: 110 *et seq.* Otto Kuntze and Tom von Post are the ferrets. Though inclined to magnify and multiply the errors, which they figure at 9315 (!), they have done a service for bibliographic work which will doubtless save users of the *Pflanzenfamilien* some hours and much bad temper.—C. R. B.

<sup>8</sup> Jour. Phys. Chem. 4: 553-569. 1900.    <sup>9</sup> Jour. Phys. Chem. 4: 533-537. 1900.

<sup>10</sup> Atti Instituto Botanico de Pavia N. S. 7: (1-21). 1899.

## NEWS.

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MR. CYRUS A. KING, formerly of Summerville, Mass., has been appointed instructor in botany in Indiana University.

R. WILSON SMITH, Ph.D. (University of Chicago), instructor in McMaster University, Toronto, during the past year, has just been promoted to the full professorship of botany in that institution.

DR. OSCAR LOEW, late of the Department of Agriculture at Washington, has received and accepted a call to become again the professor of agricultural chemistry in the University of Tōkyō. He sailed October 8.

DR. B. M. DUGGAR, assistant cryptogamic botanist of the Agricultural Experiment Station and instructor in botany in Cornell University, has returned from a year's study in Germany and has been promoted to an assistant professorship.

PROFESSOR DR. A. B. FRANK, director of the biological section of the Imperial Sanitary Bureau, formerly director of the institute for plant physiology in the Royal Agricultural School at Berlin, and author of a well-known text-book of botany and many physiological papers, died on September 27, at the age of 62.

ABBÉ A. B. LANGLOIS died at St. Martinville, La., on August 1. His long and indefatigable study of the flora of Louisiana was prosecuted in such leisure as could be obtained in the course of his parish duties. He has supplied material most liberally for the study of specialists in all groups, and his name has been used for many new species which he discovered. His cryptogamic collections were probably left to the Catholic University at Washington, to which several years ago he gave his phanerogamic plants.

THE UNDERSIGNED requests all foreign botanical writers to send to him a copy of each of their publications, as far as possible, and especially reprints of botanical articles published in proceedings, transactions, and other journals of learned societies. This request is made in order that foreign botanical literature may be reviewed promptly for Just's *Botanischer Jahresbericht*, thus bringing the same to the early notice and general use of all botanists.—PROFESSOR DR. K. SCHUMANN, *Botanischer Museum, Berlin, W.*

THE FOLLOWING report of the sixth annual meeting of the Botanical Society of America, which was held in New York City, June 26 to 28, 1900, 1900]

has just been received from the secretary. For the reading of papers the society met in joint session with Section G of the American Association for the Advancement of Science, June 28, in room 502, Schermerhorn Hall, Columbia University. The meeting of the sections was called to order by the vice president, William Trelease, who announced the arrangements for the joint session, and called B. L. Robinson, president of the society, to the chair. The retiring president, L. M. Underwood, then read his address, "The Last Quarter: A Reminiscence, and an Outlook." The full text of the address has been printed in *Science*.

Following is the program of papers presented:

- The significance of transpiration, *C. R. Barnes*;  
Relationship and variability of the Adirondack spruce, *Charles Peck*;  
Nuclear studies on *Pellia*, *B. M. Davis*;  
On the structure of the stem of *Polytrichadelphus dendroideus*, *Mrs. E. C. Britton*;  
Observations on the group *Yuccae*, *William Trelease*;  
Spermatogenesis in the gymnosperms, *J. M. Coulter*;  
The pollen tube, and division of the generative cell in pines (by invitation of the council), *Miss M. C. Ferguson*;  
On the homologies and probable origin of the embryo-sac, *George F. Atkinson*;  
Observations on *Lessonia*, *Conway MacMillan*;  
Thigmotropism of roots, *F. C. Newcombe*;  
Starch in guard cells, *B. D. Halsted*;  
Coenogametes, *B. M. Davis*;  
The development of the archegonium, and fertilization in the hemlock spruce (by invitation of the council), *W. A. Merrill*;  
The causes operative in the formation of silage, *H. L. Russell* and *S. M. Babcock*;  
A closed circuit respiration apparatus, *H. L. Russell* and *S. M. Babcock*.

The officers for the ensuing year are: *president*, B. D. HALSTED; *vice president*, R. A. HARPER; *treasurer*, C. A. HOLLICK; *secretary*, G. F. ATKINSON; *councillors*, C. E. BESSEY, F. V. COVILLE.

An important step was taken by the society in appointing a committee to consider the best means of realizing the purposes of the society in "the advancement of botanical knowledge." Among other things this committee will consider the uses to which the accumulating funds of the society may be put. The committee will report at the next annual meeting of the society.

—GEORGE F. ATKINSON, *Secretary*.

# BOTANICAL GAZETTE

*DECEMBER, 1900*

## THE ACHROMATIC SPINDLE IN THE SPORE MOTHER CELLS OF OSMUNDA REGALIS.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY.  
XXIII.

R. WILSON SMITH.

(WITH PLATE XXII)

THE cytology of the vascular cryptogams has received scant attention at the hands of botanists, as a glance at the literature of the subject will suffice to show. Calkins (6) has described the divisions in the spore mother cells of *Pteris* and *Adiantum*, reaching the conclusion that in these plants there is a true qualitative reduction of the chromosomes, in Weismann's sense. Quite recently Calkins' view has been combated by Stevens (14), who studied the spore mother cells of *Scolopendrium*, *Cystopteris*, and *Pteris*. Osterhout's (11) article on *Equisetum* is memorable as the first complete account of the formation of a spindle uncontrolled by directive spheres or centrosomes. He found that the appearance of the spindle was heralded by a felt-work of threads about the nucleus, and that the threads later arranged themselves into a multipolar spindle, and afterwards into one characteristically bipolar. These three publications, together with that by Shaw (12) on fertilization in *Onoclea*, and those by Belajeff (2, 3, 4) and Shaw (13) which treat of the origin of the cilia of spermatozoids, make up almost the total of minute research into the cytology of the pteridophytes since the introduction of improved methods of technique.

Impelled by the meagerness of our knowledge, and by the want of accord among such papers as have been published on this subject, the writer two years ago began collecting and examining the sporangia of *Lycopodium*, *Selaginella*, and various ferns, to discover some of the forms most favorable for cytological investigation. None was found so good as *Osmunda*. All three of the species occurring in the northern United States were collected, but only *O. regalis* was found to be fixed and preserved in a condition suitable for the purpose, and it alone has received any considerable attention. For want of time a full examination of the reduction divisions was not possible, and therefore attention was centered only upon such features as it was hoped might be followed in detail in the time at disposal. Accordingly in this paper only the origin, structure, and fate of the achromatic spindle are to be considered, together with such comments upon the changes in the chromatin as seem necessary for clearness. A more extensive account of the chromatic elements of the spindle is reserved for a future occasion, after there has been opportunity for further study.

The methods of fixation, embedding, and staining require a brief mention. As fixing agents, chromo-acetic acid (1 per cent. chromic acid, 0.75 per cent. acetic acid), and Flemming's weaker solution were employed. Chloroform was used as the medium of transfer to paraffin. The sections were cut  $5\mu$  or  $10\mu$  in thickness. To differentiate the fibrillar structures most clearly, various combinations of the following stains were tried: nigrosin, Delafield's haematoxylin, erythrosin, iron-alum-haematoxylin, acid fuchsin, methyl-green, iodine-green, safranin, gentian-violet, orange G. Of these stains the two combinations which have yielded the best results are iodine-green and acid-fuchsin, which was found especially advantageous in dealing with the chromatic elements, and safranin and gentian-violet, which was most effective in bringing out the details of the achromatic figure.

The sporangia of *Osmunda* make their first appearance in the latter part of summer, and continue their growth in the autumn months. By mid autumn those of *O. cinnamomea* and



*O. Claytoniana* have reached the mother cell stage, in which condition they lie dormant during the winter. The division into spores takes place in the spring, usually in northern Indiana between April 15 and May 1. *O. regalis* does not reach so advanced a condition before winter as do the other species. Cell divisions in the sporogenous tissue occur in the spring, and it is not till after the middle of April that the mother cells are differentiated; the division into spores is effected about three weeks later.

A more exact statement will be instructive. April 6, material collected showed occasional karyokinetic figures in the sporangia. April 21, first collection in which no divisions were found in the sporogenous cells; the tapetal cells were in active multiplication. May 5, many nuclei were in synapsis, the first seen. May 10, most of the nuclei were in synapsis, but some in first division. May 13, in most cases both divisions were completed, and often no trace of the spindle remained.<sup>2</sup> This indicates that the resting and early spirem stages last for about two weeks, and the synapsis stage for three or four days; and that the two divisions are effected in quick succession within a period of two or three days. The difficulty of obtaining the various stages of the second division also affords evidence that the second division follows quickly after the first, and is completed in a much shorter time.

The resting mother cell (*fig. 1*) is angular in form, and presents a rather large nucleus. The cytoplasm is evenly distributed as a delicate network of stainable matter with irregular swellings at the points of intersection; it cannot be regarded as having the structure of foam. The nucleus has a structure similar to that of the cytoplasm, differing only in the greater coarseness of its meshes, the larger size of its granular thickenings, and its more intense reactions with stains. Two or three nucleoli are usually present. In the cytoplasm are numerous deeply staining granules of variable size, each usually lying in an unstained area, as if the granules had suffered contraction in

<sup>2</sup> It should be added that the collection of May 13 was not from the same locality as those of the earlier dates.

the process of fixation. Of the nature and function of these granules, which in staining properties resemble nucleoli, little was learned except that they are not starch. They are not peculiar to any phase of cell division, though rather more numerous in the resting mother cell, or the prophase. They cannot be extra-nuclear nucleoli, for there is no increase or diminution in their size and number contemporaneous with the dissolution or renewal of the nucleoli. Moreover, the nuclear membrane is yet unbroken, when, in the later prophase of division, the nucleoli disappear from view, and therefore, if the nucleolar substance passes out into the cytoplasm at this time, it must do so by osmosis, and any relation between it and the extra-nuclear granules must be conjectural. To the inquiry if they might not be the source, at least in part, of the material of the achromatic spindle, no satisfactory answer can be given; it may be so, but positive evidence is lacking.

The mother cells enlarge for about two weeks after they have reached their full number, gradually separating from one another and becoming more and more rounded in outline. The nuclei also grow larger and pass first into a spirem condition in which the chromatic ribbon is not a single thread but much branched and anastomosed, and later into synapsis. The details of these changes need not be considered in this paper.

Meanwhile, the cytoplasm has retained its reticulate structure. But towards the end of synapsis a change is discernible, and this change is the first indication of the future spindle. It begins as an aggregation of material immediately about the nucleus, causing this region to stain more deeply with gentian-violet. The structure of this accumulation of cytoplasm, when it is first recognizable, is so delicate as absolutely to baffle the powers of the microscope; nothing definite can be said with regard to it, except that it appears to be very finely granular, especially in preparations fixed in Flemming's solution. To a similar layer about the nucleus of the pollen mother cells of *Cobaea scandens*, Lawson (8) proposed to give the name perikaryoplasm, in virtue of its position. Since Strasburger's term

kinoplasm has the advantage of more general acceptance, it will be employed here to designate not only this differentiated layer of cytoplasm but also the spindle forming material in all its modifications.

As the kinoplasm increases in quantity, it begins to assume a somewhat definite outline and texture. It becomes distinctly granular, and the granules are often disposed in short rows which run nearly concentric with the periphery of the nucleus, but are intertangled more or less confusedly (*figs. 2, 4*). In cells which have been fixed in chromo-acetic acid a more distinct fibrous appearance is presented. In some cases the fibers appear to form a loose mat; in others they are so related as to resemble a delicate meshwork with the meshes flattened towards the nucleus. Though the appearance is such as to suggest that the fibers and meshes are only a modification of the cytoplasmic reticulum, the writer was unable to trace the steps of such a transformation. The kinoplasmic material, both in its earliest form and in that assumed after cell division before its final re-transformation into reticulate cytoplasm, could not be distinguished as either reticular or fibrillar, but was very indefinitely granular.

The reader will not fail to discover the similarity of these conditions to those described by Osterhout (11), as the preparatory steps of spindle formation in the spore mother cells of *Equisetum*, although in *Equisetum* no layer of granular matter was observed. But the similarity stops here. The spindle in *Osmunda* does not pass through a multipolar stage, nor is there at any time a zone of radiating fibers about the nucleus, such as were seen by Osterhout in *Equisetum*, and by Belajeff (1), Mot-tier (9), Lawson (8), and others in the pollen mother cells of various seed plants. Tripolar spindles, though occasionally met with (*fig. 10*), were of so rare occurrence that their beginning or fate could not be traced, and it is certain they are not normal stages in the development of the spindle.

The changes in the outline of the kinoplasmic mass are very simple and easy to follow. When it has become distinctly fibrillar in structure its fibers, all the time increasing in quantity at

the expense of the outer cytoplasm, begin to collect in greater abundance at opposite sides of the nucleus and to be pushed up into dome shaped prominences. This condition is very well shown in *fig. 3*. The threads of granules run nearly tangential to the nucleus, extending out some distance at the side, and it is obvious even at this time that the axis and the bipolarity of the spindle are already determined. It is rarely the case, however, that both poles are equally prominent from the beginning; commonly one develops considerably in advance of the other. A curious relation between the position of the chromatin in synapsis and the first formed pole of the spindle was observed so frequently that it cannot be regarded as accidental. In elongated cells in which the nucleus is situated excentrically, the first pole of the spindle is formed on that side of the nucleus where there is the greatest amount of cytoplasm, while in synapsis the chromatin was almost invariably observed to be gathered within the nucleus to the side farthest from the center of the cell; that is, the chromatin in synapsis and the kinoplasm are gathered towards opposite ends of the cell. Such a relation between chromatin and kinoplasm may be true of all the mother cells, but it can be traced only in those cells which have the distinction of a long and a short axis, since the polarity of the spindle is not apparent until some time subsequent to synapsis. These peculiarities will be referred to again when the second division is considered. Every care was taken to avoid the illusion into which an observer might be misled by the effects of irregularity of infiltration of the fixing or other reagents, and the conclusion was reached that these relations have a real existence and significance in the living cell.

The writer inadvertently had an excellent opportunity to examine a condition of the cell somewhat resembling synapsis. Finding that the young sporangia, which are ensheathed in a close covering of hairs, did not sink readily in the killing fluid, he first moistened some of them for a moment with alcohol. They sank immediately, but afterwards turned out to be quite unfit for study. It was perfectly easy to trace the path of the

invading alcohol. Both cytoplasm and nucleoplasm were pushed forward; but no one could mistake such a condition for synapsis if he had once become acquainted with the latter. Contrary to what might be looked for, it was the resting cell which suffered most by this treatment; the spindle fibers, the chromosomes, and the spirem thread were not seriously affected.

It will now be necessary to refer briefly to intranuclear changes. The chromatic material, emerging from synapsis, gradually unrolls and extends itself within the nuclear cavity into a much coiled spirem, apparently of one continuous thread, for no ends can be seen in uncut parts of the nucleus. The spirem shortens and thickens, and after a time is segmented into long irregular chromosomes, which continue the shortening and thickening process already begun in the spirem. It is easy to see that many of the chromosomes are split longitudinally into pairs, and the two parts of a pair are often twisted loosely about each other. They take up a peripheral position in the nucleus, being apparently in close contact with the nuclear membrane. The halves usually remain attached to each other for a time, giving rise to Xs and Ys, or loops (as in *fig. 5*), according to the mode of attachment. But while the shortening is still going on, some of the pairs fall apart; otherwise how can the number of chromosomes shown in *fig. 7* be accounted for, if Strasburger's (15) estimate of twelve<sup>2</sup> be correct? This number has been counted in a few cases in uncut nuclei of the age shown in *fig. 5*. and also in polar views of the late anaphase in which twelve daughter chromosomes have been seen symmetrically grouped about the pole. But in nuclei of the condition shown in *fig. 7* the number of separate chromatin masses is quite variable, fourteen, fifteen, or sixteen being most common, and even as many as twenty have been counted.<sup>3</sup> If these numbers indicate a falling apart of some of the chromosome pairs, there must be a subsequent reunion before their arrangement into such an equatorial plate as that shown in *fig. 8*, in which of the twelve chromatic

<sup>2</sup> See note at the end of the paper.

<sup>3</sup> Guignard counted as many as twenty-two.

masses eleven are clearly double. Whether in the case of the larger numbers all the groups represent pairs, or whether some of them are single chromosomes, has not been determined. It is by no means certain, however, that the number of chromosomes in the equatorial plate is so constant as theories of nuclear division which are accepted at the present time require us to assume.

There is a corresponding development of the spindle fibers coincident with the maturation of the chromosomes. The spindle acquires a more distinctly bipolar form, and its fibers, which become coarser and longer, run continuously from pole to pole. During the prophase they have the appearance of knotted cords or strings of loose beads. These features can be seen in *fig. 5*, and also in *fig. 6*, which represents a section of a cell cut sufficiently deep to remove one chromosome and part of the equatorial region of the spindle. But in the metaphase and anaphase the knotted appearance is no longer recognizable. The fibers then appear as stout uniform threads or rods (*figs. 9, 10*). The same statement can be made of the second division (*figs. 17, 18*); that is to say, the fibers at the time when they are functioning in the separation of the chromosomes are of uniform diameter and texture. They are rows of granules at all other times, either when they are disappearing or in process of formation.

It is evident that the achromatic spindle is wholly of cytoplasmic origin. If any nuclear material takes part in its formation, it can do so only after passing through the nuclear membrane. There can be no direct union of linin or other nuclear substance with the kinoplasm, for in nuclei, as far advanced in the prophase as those shown in *figs. 5* and *7*, the chromosomes are the only stainable constituent remaining; no fibers of any kind can be made out, nor any trace of the nucleolus.

A comparison of the shape of the spindle, as seen just before and after the dissolution of the nuclear membrane, suggests that this period is marked by a sudden change. While the nuclear membrane is still present, the spindle has its poles

rounded and ill defined, and its breadth relatively great in comparison with its length. But in all cells from which the nuclear membrane has disappeared, the spindle is seen to be considerably narrower, and at the same time longer and more sharply pointed. To explain these phenomena it seems necessary to suppose that a pressure, exerted by the spindle fibers upon the nucleus, is sustained by the nuclear membrane, and that when the membrane finally gives way, its collapse is attended by a sudden diminution of the diameter of the spindle (*figs. 7, 8*) together with a corresponding increase in length. At the same time, the pressure of the fibers crowds the chromosomes close together. If the fibers be conceived of as curved elastic rods, this action becomes intelligible; that they are rigid enough to exert pressure is probable from the fact that the second spindles frequently cause a widening of the ends of the mother cell, pushing out the wall so as to give it the form in section of a figure 8.

One effect of the sudden collapse of the nucleus is that the chromosomes, which were all necessarily on the inner side of the spindle fibers, are most of them forced to the outside. No distinction of central and mantle fibers was possible, though very great care was exercised at this point of the investigation. It is clear from *fig. 9* that the original fibers, which run meridionally, still remain so after the separation of the chromosomes. Repeated searching under a magnification of 2250 diameters failed to reveal any fibers other than those which run from pole to pole, or any thickening of the fibers towards the poles, as if some portion attached to the chromosome were undergoing contraction. How then are the chromosomes propelled? Inasmuch as there are no fibers discoverable by which they can be pulled, is it not possible that they have a power of motion in themselves?

This hypothesis is not absurd. It is quite as reasonable to assume an automobility of the chromosomes as a contractility of the spindle fibers. The initiatory separation of the chromosomes into pairs by longitudinal fission implies a power of movement

which is entirely independent of external tension; and the same inference is possible from the gradual shortening of the spirem to form the chromosomes, and from the re-expansion of the chromatin in the daughter nuclei.

Meanwhile, the outer region of the cytoplasm loses its reticulate structure (still evident in *figs. 3, 4*), and passing through a condition in which it appears to be a diffusely staining mass becomes converted into granules, as in *figs. 8 and 9*. This change, which is comparable to that preceding the organization of the kinoplasm into fibrillae, advances gradually from the spindle outwards towards the cell wall. Directly after the metaphase the granules of this outer zone arrange themselves into rows stretching from the poles out towards the equatorial region of the cell. This condition is shown in *fig. 9*, and again in the second division in *figs. 17 and 18*. These rows of granules, which soon become more threadlike, will be spoken of as *secondary* fibers, to distinguish them from the primary fibers that form the central framework of the spindle. They are not mantle fibers, for they do not enter into connection with the chromosomes, and are only just coming into existence when the latter begin to withdraw from the equatorial plate towards the poles. The secondary fibers multiply rapidly, and by the end of the anaphase fill all the outer part of the cell; in fact all the cytoplasm seems to have been exhausted in producing them.

Almost immediately there begins a breaking down of the spindle fibers, indicated in the first place by their reassuming the appearance of dotted threads. In all cases the primary spindle is the first to disappear; then the secondary fibers break down and all the stainable cytoplasm is of granular texture (*figs. 11, 13*). A cell plate is first formed, which however comes to nothing; it can be traced through *figs. 12-18, 21*.

Though the second division follows quickly after the first, there is a sufficient interval between to allow the formation of a nuclear membrane and the partial reorganization of the chromatin. The chromosomes after assuming a very symmetrical pattern about the poles unite into a chromatic mass which



takes the shape of a shallow cup with the concavity towards the center of the cell.

If there is any synapsis in the second division, it is represented by the case of *fig. 13*. It will be interesting to compare this with the conditions prevailing at synapsis of the mother cell. In *fig. 13*, as in the synapsis formerly described, the chromatin is bunched on the side of the cell most remote from the greater mass of kinoplasm. In this case it is easy to understand how these conditions have arisen from those preexisting. Whether the relation of the chromatin to the kinoplasm is determined in the mother cell in the same manner, by the position of the last preceding spindle, it is impossible to say, on account of the long rest which the mother cell passes through. If it is so determined, it argues strongly for the organic continuity of the kinoplasm, or at least of some specific substance which retains a definite position in the cell and reacts on the cytoplasm so as to cause it, at the proper time, to be transformed into a fibrillar texture and organize the spindle. It should be remembered, however, that the first visible signs of approaching division in the mother cell are intranuclear, hence so far as we can judge from appearances, if the stimulus to division originates from some special region or structure, and not from the activity of the cell as a unit, we must assume that the primary impetus proceeds from the chromatin.

Apparently the preparations within the nucleus for the second division, and those in the cytoplasm, do not always progress with equal rapidity relatively to each other, for one may meet with cases in which the second spindles are well formed, while the nuclear membrane is still unbroken, and others in which the nuclear membrane is gone and the chromosomes of the second division well organized, while the spindle is still very imperfect. Invariably, however, the development of the second spindle is preceded by an accumulation of material along those borders of the daughter nuclei which were in contact with the old spindle. The fibers first appear in this region also, and the spindle as seen in sections across this part of the cell is bipolar from the

beginning. The poles are not diametrically opposite each other, but both lie on one side of the nucleus, as shown in *fig. 15*<sup>1</sup>. Invariably, too, the axes of the second spindle are at right angles to that of the first; that is, though they may be at any angle with reference to each other, they always lie in planes which are parallel to the first cell plate (*figs. 15-18*). In the preparations selected for drawing, the second spindles are either parallel (*figs. 14-17, 21*), or at right angles (*fig. 18*). It must not be understood, however, that these cases are typical in this respect; they were chosen because in them both the spindles are in view at once.

Undoubtedly the material used for the building up of the second spindle is obtained from the disintegration of the first, not as fibers, however, but as granules; and in this abundance of granular matter ready formed in the cell, we may perhaps see an explanation of the rapidity of the second division.

The phenomena attending the metaphase of the second divisions agree completely with those of the first division. There is the same absence of mantle fibers (*figs. 17, 18*), the same continuity of the fibers from pole to pole (*fig. 19*), the same excess of fibers over the number of chromosomes (*figs. 18, 19*). In the anaphase there is the same elongation of the chromosomes into crooked or lobulated rods, the same beaded appearance of the fibers as soon as the chromosomes have passed to the poles (*fig. 21*), and the same development of secondary fibers which run from the poles towards the equator.

The beginning of the secondary fibers is shown in *fig. 19*; they are more abundant and longer in *figs. 20* and *21*, in which it is seen that those put out from the poles of one spindle meet those from the other. The secondary fibers so meeting unite by their ends into continuous threads, which connect the four poles of the primary spindles. In this way four secondary spindles are formed; and thus the four daughter nuclei are now joined by six symmetrically placed spindles, of which two are primary and four secondary. Meantime, there has been a slight rotation of the primary spindles so that their planes, which were at

first parallel to one another, come to lie at an angle of  $60^\circ$ . This results in placing the four daughter nuclei equidistant from one another in the tetrahedral arrangement which is characteristic of fern spores. Whether the rotation is brought about by the action of the secondary fibers, or by some other influence acting on the nuclei, could not be determined. In *fig. 21* two primary spindles are shown, and some of the secondary fibers not yet united by their extremities. In *fig. 22* are one primary spindle (that on the left side) and two secondary; the axes of the other three spindles would make angles of  $60^\circ$  with these or with the plane of the paper.

The disappearance of the spindle has been followed with great care in order to discover, if possible, in what manner the kinoplasm is metamorphosed into ordinary cytoplasm. It was found that the middle fibers of all the spindles are the first to disintegrate, the material being used up in part to build the cell plate. After the spindles are no longer distinguishable, a considerable amount of granular or amorphous matter remains on the inner side of each nucleus (*fig. 24*); finally this disappears and the cytoplasm assumes a reticulate structure throughout (*fig. 25*). All attempts to identify the granules of kinoplasm with microsomata, or the fibrous matter of the spindle with the cytoplasmic reticulations, were unsuccessful. The fibers of the spindle are composed of modified cytoplasm, which in the transformation loses its characteristic structure and becomes first structureless in appearance, then granular, then fibrous, and in returning to its normal condition reverses these steps.

#### SUMMARY.

The achromatic spindle originates wholly from cytoplasmic material (kinoplasm) which accumulates about the nucleus in the synapsis or spirem stage in the form of an indefinitely granular mass of stainable matter.

The kinoplasm becomes distinctly granular; then the granules arrange themselves into short rows concentric with the nuclear membrane; finally the rows of granules are massed in

greatest abundance on opposite sides of the nucleus, foreshadowing the development of a bipolar spindle.

Usually one pole is formed considerably in advance of the other; and in cells cut parallel to their long axis, it can be seen that the first pole (the greatest accumulation of kinoplasm) is on the side of the nucleus remote from the chromatic mass of synapsis.

The spindle is bipolar from the beginning. Nĕmec's (10) generalization, therefore, that sporogenous cells as compared with vegetative cells are characterized by their spindles passing through a multipolar phase, does not hold good of *Osmunda*.

The fully formed spindle shows no distinction of central and mantle fibers, and no bodies which can be interpreted as centrospheres; all the fibers run from pole to pole.

The dissolution of the nuclear membrane is attended by a sudden narrowing of the spindle and a corresponding increase in length.

During the anaphase new (secondary) fibers, not to be confounded with mantle fibers, are put forth about the poles and meet in the equatorial region of the cell.

In the late anaphase the primary fibers, and soon after them the secondary fibers, begin to disintegrate, taking the appearance of beaded threads, and then of granules; at this time all of the stainable cytoplasm of the cell appears granular in texture.

The spindles of the second division at first have their axes parallel to the first cell plate. They are constructed out of the granular products arising from the disintegration of the first spindle.

The phenomena of the second spindles exactly repeat those of the first, except that four secondary spindles are formed by the union of the secondary fibers put forth during the anaphase.

The primary spindles become rotated about each other so as to bring the four daughter nuclei into the tetrahedral arrangement.

Cell plates are formed across the six spindles (two primary and four secondary), and in connection with them the separating walls of the spores are laid down.

Such a relation between the fibrillae of the kinoplasm and the cytoplasmic reticulum as Blackman (5) reports in *Pinus*, and Lawson (8) in *Cobaea scandens*, could not be verified. Between well developed spindle and cytoplasm are the three stages, (1) dotted fibers, (2) granules, (3) amorphous kinoplasm (structure too delicate for the microscope to reveal). The same phases in reverse order were traced in the first formation of the spindle.

This investigation was conducted in the Hull Botanical Laboratory of the University of Chicago during the spring and summer of 1899. The writer, while assuming full responsibility for the views expressed, takes pleasure in acknowledging his indebtedness to the members of the Botanical Staff for their courtesy and encouragement, and especially to Dr. Bradley M. Davis, under whose more immediate direction the work was undertaken.

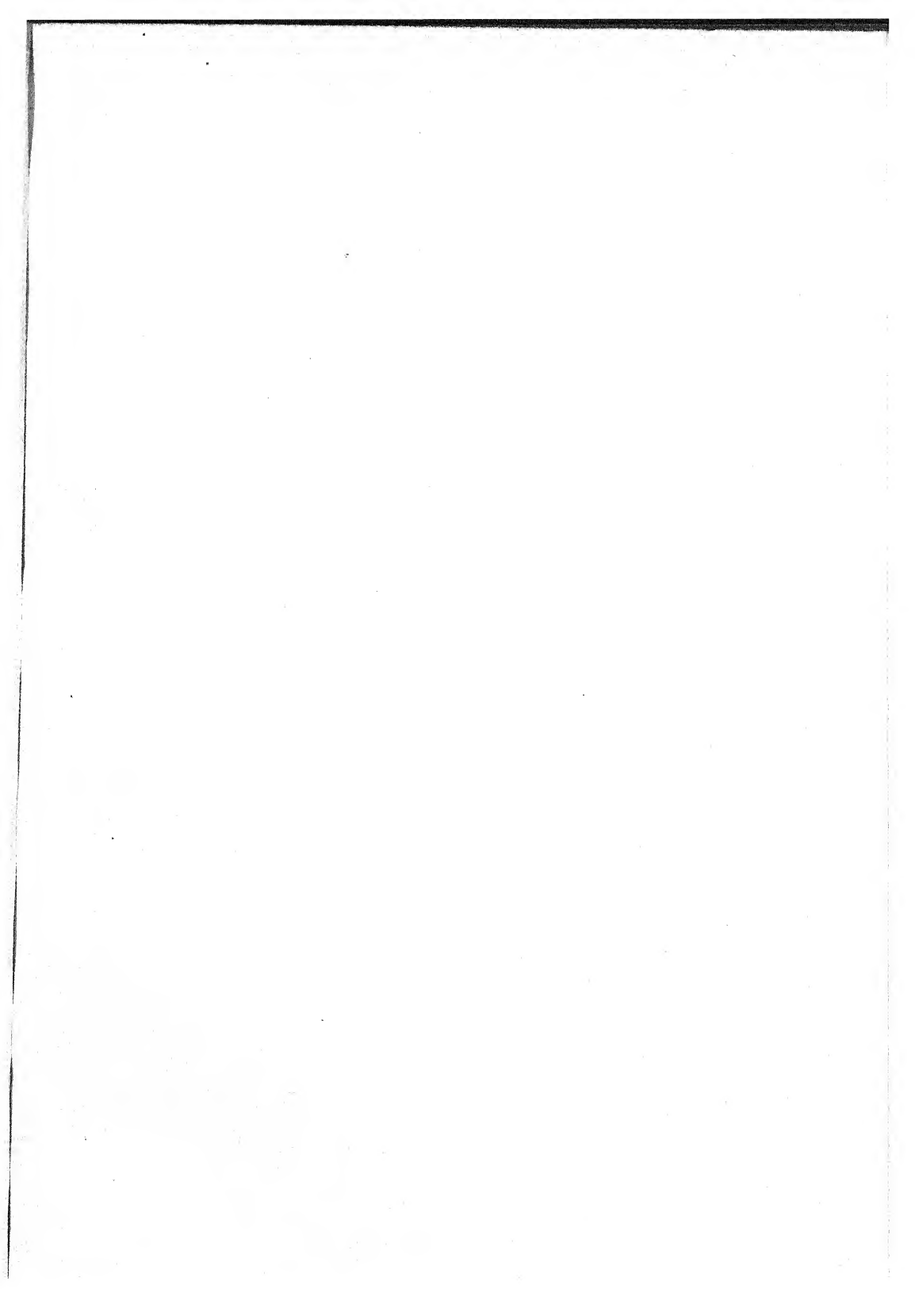
\*\*\* Since the foregoing account was written (in February 1900), a comprehensive work on karyokinetic problems (15a) has been issued by Strasburger, in which, among other topics, he discusses the divisions of the spore mother cells of *Osmunda*, and the general formation of achromatic spindles in plants. He distinguishes two types of spindles, those possessing centrosomes and those without such controlling centers. The latter, which are characteristic of higher plants, are again subdivided into multipolar polyarch spindles, such as those of the spore mother cells of *Equisetum*, and multipolar diarch spindles, such as are common in various spermatophyte root-tips; and he seeks to minimize the divergence between these two extreme types of multipolar spindles by instancing numerous intergradations between them. My observations on *Osmunda* accord well with his view; for though I have described the spindle as bipolar from the beginning, its appearance soon after its inception is such that it may very properly be termed multipolar diarch. Strasburger recognizes in the spindle two kinds of fibers, which, having the same origin and reaction to stains, he prefers to call not central and mantle fibers, but supporting and attracting fibers (*Stützfasern* and *Zugfasern*). He pronounces against a power of movement in the chromosomes themselves, and attributes their withdrawal from the equatorial plate to the action of the attraction fibers. This conclusion is quite at variance with that expressed in the preceding pages. To the view that the nucleolus is used up to help complete the achromatic spindle, my observations, though not contradictory, are not altogether favorable; the spindle fibers are too

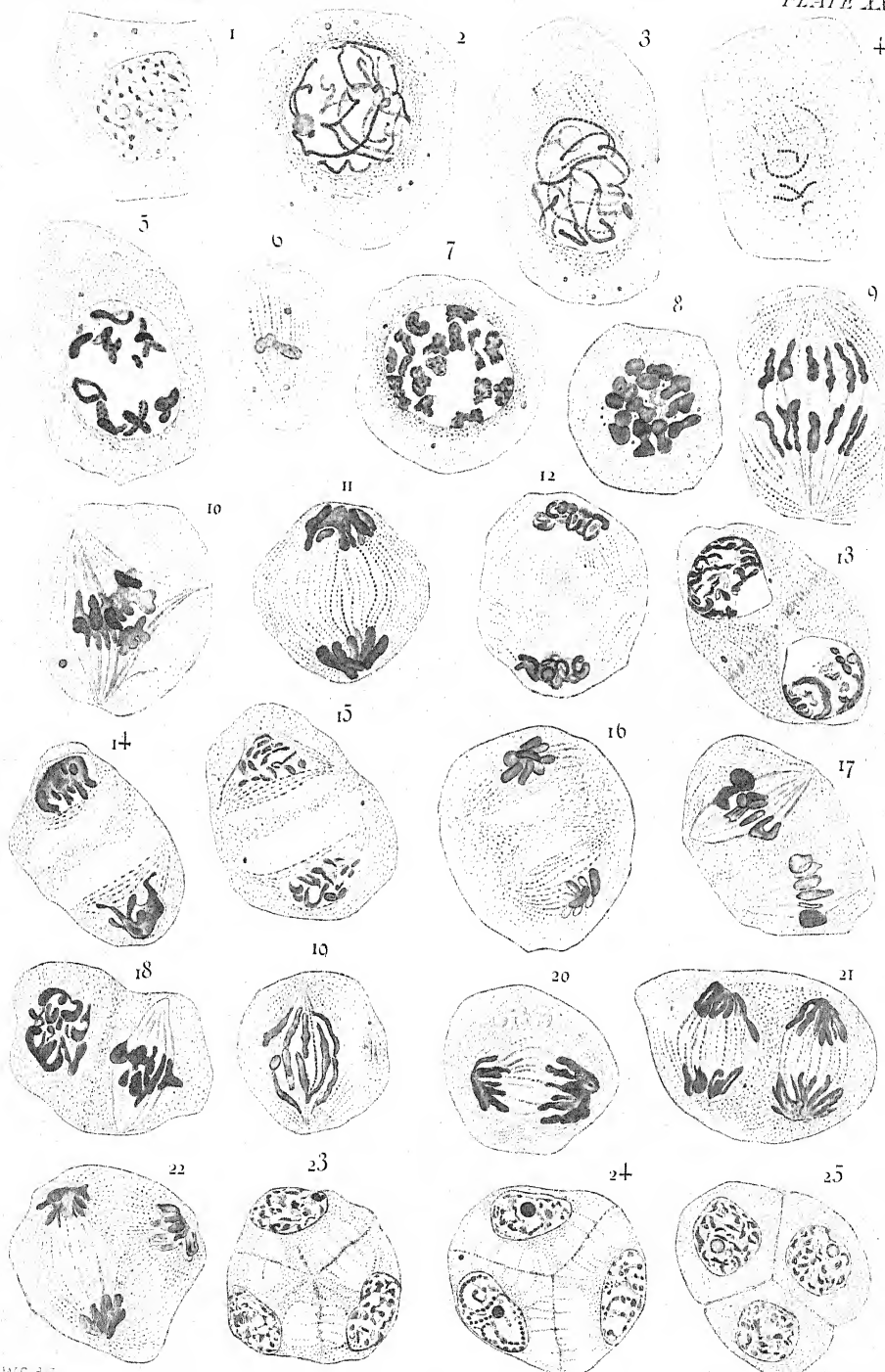
nearly complete before the disappearance of the nucleolus. In his renewed observations on *Osmunda*, Strasburger has noted the large and varying number (20-22) of chromatic groups in the spore mother cell. Each such group he regards as a chromosome pair. He finds the number of chromosomes in the prothallial cells is not so constantly twelve as he formerly (15) stated; it may reach sixteen or more. It is therefore certain that the current view as to the constancy of chromosome numbers cannot be maintained, at least as regards *Osmunda*.

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R.W.S. del.

SMITH on ACHROMATIC SPINDLE.

Lith. Anst. E.A. Pott & Co. Leipzig



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#### EXPLANATION OF PLATE XXII.

The figures were outlined by the aid of an Abbé camera under the magnification given by a Zeiss apochromatic oil immersion lens 2<sup>mm</sup> aper. 1.30, in combination with a Zeiss compensation ocular no. 8. In all cases the preparations were studied with the higher oculars, 12 and 18.

FIG. 1. Mother cell with resting nucleus.

FIG. 2. Mother cell with nucleus in spirem stage, and the kinoplasm distinctly granular and fibrillar.

FIG. 3. The same, somewhat more advanced, showing the bipolarity of the kinoplasmic mass.

FIG. 4. A section which cuts off only a portion of the nucleus.

FIG. 5. Chromosome stage.

FIG. 6. Portion of a cell, showing part of the equatorial region of the spindle.

FIG. 7. Later chromosome stage.

FIG. 8. Chromosomes in equatorial plate after the disappearance of the nuclear membrane.

FIG. 9. Anaphase, showing continuity of the primary fibers from pole to pole, and secondary fibers extending out from the poles.

FIG. 10. A tripolar spindle.

FIG. 11. Late anaphase.

FIG. 12. Late anaphase.

FIG. 13. Telophase; the primary spindle has quite disappeared.

FIGS. 14-16. Early stages of second division.

FIGS. 17-18. Metaphase of second division.

FIG. 19. Anaphase; secondary fibers beginning to form.

FIGS. 20-21. Late anaphase.

FIG. 22. Late anaphase; one of the primary spindles on the left side; the two other spindles shown have been formed out of the secondary fibers.

FIGS. 23-25. Successive telophases.

STUDIES ON CHROMOGENIC BACTERIA. I.  
NOTES ON THE PIGMENT OF BACILLUS POLYCHROMOGENES.

E. M. CHAMOT and G. THIRY.

(WITH SIXTEEN FIGURES)

THE *Bacillus polychromogenes* was first isolated from a well water of Nancy by Macé<sup>1</sup> in 1894, and since its first discovery he has met with this same organism on five different occasions in well and conduit waters of that city. Two years after its discovery it was again isolated from the same well, and was found to possess the same characters as in the previous case. The organism was then described by one of us under the name of *Bacille polychrome*.<sup>2</sup>

These six colonies, found at different times, have varied neither in the original colonies nor on subsequent cultivation; varieties are, therefore, still unknown. Neither has it been possible to obtain variation by culture methods, for although one of the original colonies has been grown in the laboratory since 1894, part of the time in America, no change has been observed. It has also been impossible to obtain a non-chromogenic variety in spite of all attempts. It seems more than probable that this beautiful species will be met with by other investigators, and therefore, although the present article has to deal with the pigment, a few words regarding the characteristic features of the bacillus may not be out of place.

The *B. polychromogenes* was so named because of its peculiar power of giving a multiplicity of colors on ordinary culture media. On such media the organism produces at times blue, at

<sup>1</sup> MACÉ, E.: *Traité pratique de Bactériologie*. Ed. 3. 849-852. 1897. *Atlas de Bactériologie*, pl. 29.

<sup>2</sup> THIRY, G.: *Sur une bacterie produisant plusieurs couleurs*. C. R. de la Société de Biologie, 7 Nov. 1896.

Contribution a l'étude du polychromisme bactérien. *Archives d. physiol.* V. 9: 284-289. 1897.

others red, green, violet, purple, or yellow. These colors, as will be shown, can generally be controlled, thus permitting one to obtain at least part of the colors with certainty. This interesting fact has already been pointed out by the authors.<sup>3</sup> In addition to the pigment described below, colored insoluble microscopic crystals are also generally found on solid media. These crystalline aggregates are made up of irregular radiating clusters of fine needles of a deep blue color, and are probably not due to a crystallization of the pigment, but to crystals of some other substance stained by it. The composition of these crystals will be discussed in a future communication.

The organism liquefies gelatin, solidified blood serum, albumin (white of egg), fibrin, etc. In peptone solutions no indol is produced, neither does the bacillus produce gas in fermentation tubes filled with glucose or lactose peptone solutions at room temperature nor at incubator temperature.

The bacilli themselves are generally colorless, rarely they appear red or blue; in some of these latter cases the organism is uniformly stained, at others times only minute intra-bacillary granules are colored. The bacillus is polymorphic; not only does its form vary according to the nutritive media employed, but often there is great variation in form observed in different portions of the same medium. It has not been possible thus far to obtain with any degree of certainty a constant form on any culture medium, not even when employing one of definite composition (*i. e.*, a medium in which the source of nitrogen is not peptone etc., but a chemical compound of known composition such as asparagin or other bodies). In general the organism assumes the form of a short rod rounded at the ends, at other times it is spherical; again, long curved giant forms with swollen ends are seen. The bacilli are sometimes isolated, sometimes grouped (diplo-bacilli, chains). Coccus forms are likewise to be found in staphylo- and diplo- forms, or as tetrads and chains of eight cells.

<sup>3</sup> CHAMOT, E. et THIRY, G.: Bacille polychrome. Cultures et Spectre du Pigment. Communication à la Réunion Biologique de Nancy, Feb. 1898.

Usually the organism is motile, but the motion is always slow. Motility is to be seen in colored as well as in colorless individuals. It stains well by the ordinary methods and retains the stains by the method of Gram and that of Claudius. Greater morphological details would take us beyond the scope of the present note, namely, an account of some of the results of the study of the pigment produced by this bacillus on potatoes and on gelatin.

*Growth on potatoes and the pigment formed.*—On a medium as variable in composition as potatoes of different varieties and different ages of growth, it might be expected that there would be considerable variation in the colors of the pigment and nature of the growth of a chromogenic organism. The *B. polychromogenes* shows the effect of such changes in a most marked manner. Potatoes upon which this organism grows are colored variously yellow, greenish, red, violet, blue; the last color predominating but not constant. It was soon found, however, that a beautiful deep blue could be obtained, almost without fail, if the potatoes were first soaked in a dilute solution of sodium hydroxid (0.25 per cent. to 0.50 per cent.) containing a little calcium phosphate for twenty-four hours or less, depending upon the thickness of the pieces. Since this medium has served as the basis for the isolation of the pigment, and is being constantly employed by us in the study of the pigments of other chromogenes, it may not be out of place to describe our methods of preparation.

As large tubers as possible are chosen, such as are known to become mealy and porous on boiling. They are well washed in cold water, using a brush to aid in cleansing, and are dropped into boiling water with the skins on, and boiled till just cooked through. The water is then poured off, the potatoes allowed to cool somewhat, peeled, cut into slices 1 to 2<sup>cm</sup> thick, and dropped into a dilute solution of sodium hydroxid, where they remain about eighteen hours. The supernatant liquid is then poured off, the slices drained and transferred to glass boxes 100<sup>mm</sup> in diameter and 49 to 50<sup>mm</sup> deep, with loosely fitting covers (in other words deep Petri dishes), a little water is added, and the

medium sterilized three days in succession in streaming steam.

When an active culture of *B. polychromogenes* is inoculated upon such a piece of potato, a deep blue very soluble pigment is produced, which diffuses slowly through the whole mass of the medium, coloring the latter an intense indigo blue. In from ten to fifteen days the coloring matter has penetrated the entire mass, and has colored it uniformly and so intensely that the slice of potato appears almost black. The more porous the potato, the more thoroughly and uniformly is it colored, since the pigment is reduced in the absence of air. For some time the color remains of the same intensity, then it becomes violet or purple, and then, owing either to the organism being no longer able to produce pigment to replace that being reduced by the various reducing substances present in the potato, or owing to the production of reducing agents by the culture itself, the color begins to fade. Decolorization proceeds more and more rapidly, the color at the surface of the medium exposed to the air being the last to disappear. The culture medium finally assumes a dirty brown color. Generally, cutting up the potato into thin slices and exposing them to the air leads to the production of a blue again by oxidation, providing the culture is not too old.

The blue pigment is very soluble in water, quite soluble in *dilute* alcohol, insoluble in strong alcohol, in ether, chloroform, benzine, etc. Water, therefore, is the best solvent; but unfortunately extraction with water removes such an amount of reducing substances that it was found necessary to employ dilute alcohol, the strength of the latter varying with the moisture present in the culture to be extracted.

In order to extract the pigment, the piece of culture medium is cut into thin slices and exposed to the air for a short time in order that as much of the blue pigment as possible shall be formed. Dilute alcohol is then poured over the material and allowed to act for some hours, the blue alcoholic solution is poured off, and a fresh addition of the solvent is made. This is repeated as long as any coloring matter can be removed. The

alcoholic solution of the pigment is then filtered through a carefully cleaned bacterial filter (Chamberland, Kitasato, d'Arsonval, or others). The first portions of the filtrate are rejected, owing to the fact that a chemical action takes place at first, doubtless owing to air present in the pores of the tubes; the result is the production of a beautiful purple-red liquid. After some 50<sup>cc</sup>, more or less, have passed, the filtrate passes unchanged in color. It is then evaporated to a syrupy consistence at 50° to 60° C.; a higher temperature leads to reduction and decomposition of the pigment at this stage, owing to the presence of large amounts of sugars extracted from the potatoes. The thick deep-blue liquor is precipitated with strong alcohol (about 98 per cent.), the supernatant liquid poured off, and the precipitated pigment dissolved in a very small amount of distilled water and again reprecipitated with alcohol. This process is repeated as long as the alcohol seems to extract anything from the pigment. At this point the pigment is precipitated in such a finely divided condition that it refuses to settle completely, and cannot be retained on filter paper; a very small bacterial filter is therefore employed for the separations. The material is then carefully removed from the filter tube and dried. This dry amorphous powder has a grayish-blue color, and is completely soluble in water to a beautiful pure-blue color. Although we have reason to believe that it is still impure, the amount of impurity is doubtless so small that the pigment thus separated can be used as the basis of comparison, and for the reactions given in this paper. It cannot be made to crystallize, and is insoluble in all ordinary solvents, such as ether, petroleum ether, benzene, chloroform, amyl alcohol, etc.

If the blue aqueous solution is treated with a trace of acid, the blue is changed to a violet, a trifle more acid leads to the production of a beautiful purple (royal purple). An excess of acid gives rise to a red with more or less of a purple tint. So sensitive is the compound to acids that carbon dioxid causes a change of color. It was at first thought that when organic acids were employed, a color change resulted which was different

from that produced by inorganic acids. Later experiments seem to indicate that this is more probably due to a difference of intensity of action.

Ammonium hydroxid causes a change similar to that produced by acids, but the red color in this case is of a different tint from that obtained with the latter. The addition of acids to the purple-red ammoniacal solution restores the blue color, but if the acids are added in great excess the red tint of acid solutions results. It is worthy of note that a decided excess is necessary.

Fixed alkalies (potassium, sodium, barium hydroxids), in small amount, first produce a violet tint; if a little more of the reagent is added a pure blue results, but the color is somewhat paler than that of the original solution. When added in excess, the fixed alkalies give rise to a grass green solution. When the pigment has not been carefully purified, or when filtrates directly from a culture are employed, the change to green is much more rapid, and the amount of alkali required for its production is less. If the pigment is quite free from foreign bodies the green is rather persistent, but when impure rapidly fades away, leaving a yellowish liquid. The process of decolorization begins at the bottom and gradually extends upwards until the surface is reached; here, being in contact with the oxygen of the air, the color persists. If the yellowish alkaline liquid be shaken with air it immediately turns green, then blue; and if the agitation be continued there results a blue solution of almost the same intensity as the original. Allowed to stand undisturbed a reverse change is observed, namely, rapid decolorization passing through a green. The blue can be restored even after several days by shaking with air. The addition of alcohol to the yellowish solution produces a dirty yellow precipitate which turns blue the instant it comes in contact with oxygen. This phenomenon explains why it is that porous potatoes yield most pigment, and why cutting the colored medium into thin slices and exposing it to air before extracting, gives a larger quantity of the coloring matter; for it seems to be obvious that we have to do here with a case of oxidation.

Fixed alkalies added to the red solution resulting from the action of acids on the blue first restore the blue color, then, if in excess, produce a green, which in turn disappears as has just been described; and in like manner shaking with air or addition of hydrogen peroxid restores the blue. From neither acid nor from alkaline solution will solvents such as petroleum ether, ether, chloroform, benzene, amyl alcohol, etc., extract any coloring matter.

As to the important question whether the organism produces the blue pigment or a compound which turns blue in air, the writers do not yet feel justified in advancing an opinion.

When the clear blue solution (obtained by dissolving in water the coloring matter isolated from potatoes by the method described above) is placed before the spectroscope, a fairly well defined absorption band is seen in the neighborhood of the Dline. The maximum intensity of this absorption band has approximately a wave-length of  $\lambda=594$ , in the case of the purest pigment thus far obtained; its width and intensity varies, naturally, with the concentration and thickness of layer of the solution examined. There is also a slight darkening of the spectrum in the red, and a similar cutting off in the blue extending to the far violet. In the red this absorption seems to begin somewhere from  $\lambda=680$  to  $\lambda=690$ , but is so gradual that no satisfactory measurements can be made. In the green, blue, and violet the increasing absorption is so gradual that no reliable decision can be made as to just where the absorption begins. *Fig. 1* gives the absorption spectrum of solutions 10<sup>mm</sup> thick, containing 1<sup>gm</sup> per liter of the purest pigment obtained; *fig. 2*, same solutions in layers 25<sup>mm</sup> thick; *fig. 3*, same pigment in solutions of 2<sup>gm</sup> per liter, examined in layers 10<sup>mm</sup> thick. In 25<sup>mm</sup> layers the absorption bands of these last solutions are too intense to permit of their being represented on the same scale as those figured. Blue solutions obtained by mere filtration of water extracts of colored potatoes usually show more marked absorption in the red and violet ends of the spectrum than do solutions of the pigment separated as previously described. *Fig. 16* shows the spectrum



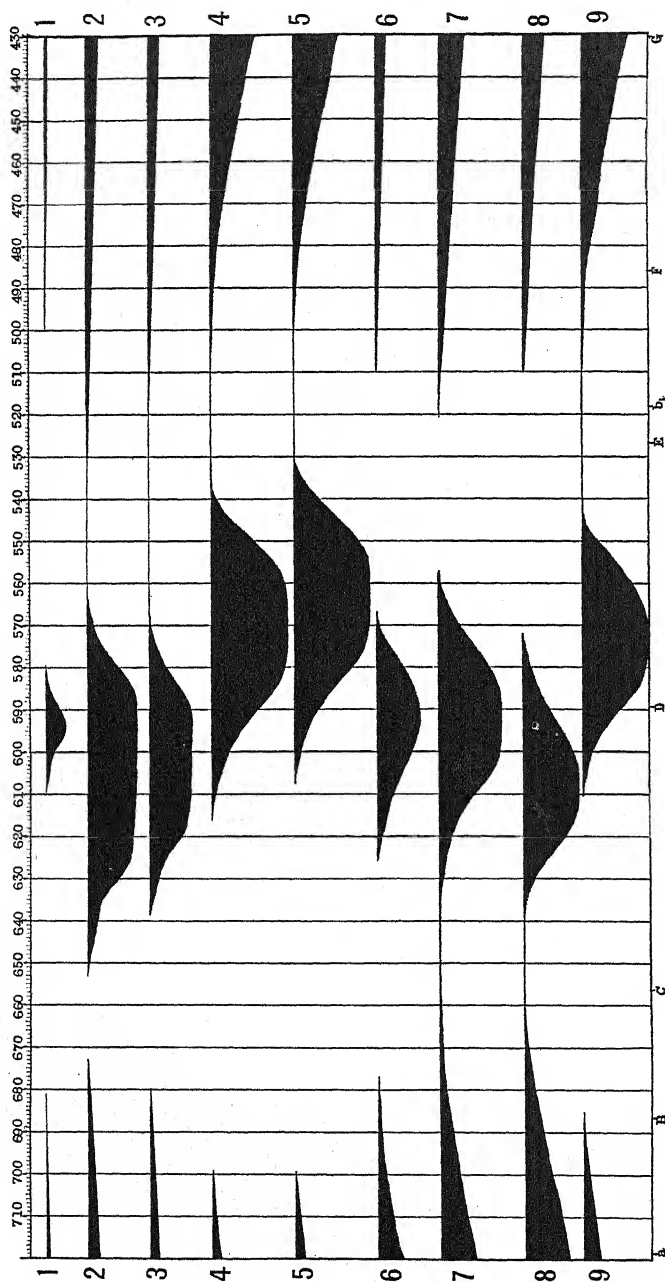


FIG. 1. One gram of pigment per liter in layer 10<sup>mm</sup> thick.—FIG. 2. One gram of pigment per liter in layer 25<sup>mm</sup> thick.—FIG. 3. Two grams pigment per liter in layer 10<sup>mm</sup> thick.—FIG. 4. Two grams per liter, 10<sup>mm</sup> layer, plus acetic acid.—FIG. 5. Two grams per liter, 10<sup>mm</sup> layer, plus hydrochloric acid.—FIG. 6. Two grams per liter, 10<sup>mm</sup> layer, plus ammonium hydroxid.—FIG. 7. Two grams per liter, 25<sup>mm</sup> layer, plus sodium hydroxid until violet color results.—FIG. 8. Filtered aqueous extract of potato, plus sodium hydroxid 10<sup>mm</sup> layer. Color intensity about equal to 2 grams pigment per liter.—FIG. 9. Same solution as that used for fig. 8 after addition of acetic acid. 10<sup>mm</sup> layer.

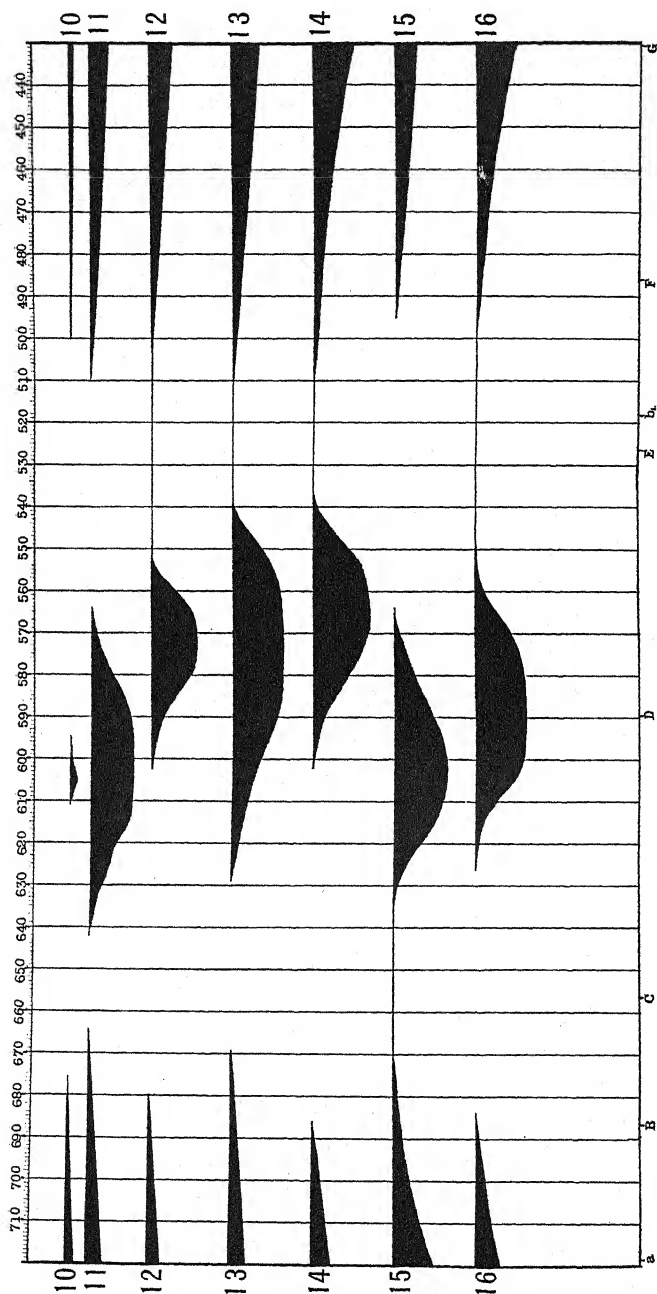


FIG. 10. Two grams pigment per liter, 25<sup>mm</sup> layer, plus sodium hydroxid in excess.—FIG. 11. Green dichroic solution from gelatin culture three days old.—FIG. 12. Same solution as that used for fig. 11 after addition of acetic acid. 10<sup>mm</sup> layer as in fig. 11.—FIG. 13. Violet solution from growth on gelatin containing glucose.—FIG. 14. Same solution as that used for fig. 13 after addition of acetic acid.—FIG. 15. Same solution as fig. 13 after sodium hydroxid added in excess and mixture shaken with air until blue.—FIG. 16. Filtered aqueous extract from potatoes. 10<sup>mm</sup> layer. Color intensity about equal to that of 2 grams pigment per liter.

obtained when  $10^{\text{mm}}$  layers of such filtrates are examined, after having been diluted to a color intensity approximately equal to  $2^{\text{gm}}$  per liter of purified pigment.

The addition of acids to the blue solutions causes an immediate displacement of the main band toward the violet, and increases its intensity at least twofold. The maximum intensity, of the main absorption band, of the now purple-red solutions is about  $\lambda=570$ . The absorption in the red seems to be diminished, but that in the blue and violet slightly increased. In *fig. 4*, the effect of acetic acid is shown; in *fig. 5* that of hydrochloric acid. In each case solutions of  $2^{\text{gm}}$  of the isolated pigment per liter, in layers  $10^{\text{mm}}$  thick, were employed. *Fig. 9* shows the effect of acetic acid on the solutions used in *fig. 16*.

Ammonium hydroxid added in excess to the pure blue solution causes but little displacement of the main band, but greatly reduces its intensity, as also that of the bands in red and violet. This is not the result of dilution alone, however, since the addition of a corresponding volume of water gives no similar reduction in the intensities of the bands. *Fig. 6* shows the action of this reagent in slight excess on solutions of  $2^{\text{gm}}$  of the pigment per liter, when examined in layers  $10^{\text{mm}}$  in thickness.

Fixed alkalis when added in *very* small amount first produce a violet color. Such solutions yield an absorption spectrum shown in *fig. 7*, in which sodium hydroxid has been added to a  $25^{\text{mm}}$  layer of a  $2^{\text{gm}}$  per liter solution of pigment. Added in excess until a green results, the absorption band at D is almost completely destroyed, and the other bands about equally reduced in intensity. This effect is shown in *fig. 10*, where solid sodium hydroxid (to avoid dilution) has been added to solutions similar to those used for *fig. 7*. If the alkaline solution be shaken with air until it turns blue, a dark absorption band again appears, but not in the same position as in the original solutions, for it has been displaced toward the red end of the spectrum; its maximum intensity now falls between  $\lambda=605$  and  $\lambda=610$ . This change in position is indicated in *fig. 6*, solutions similar to those used in *fig. 16* being employed.

*Growth on gelatin.*—Grown on gelatin of standard composition, liquefaction results and a green color is produced which gradually diffuses throughout the medium, the color being most intense near the surface. Generally after a few days a distinct red dichroism (fluorescence?) appears which increases with age.<sup>4</sup>

For the production of the coloring matter in large amount the following method is employed. A 10 per cent. solution of gelatin is made nutrient by adding 1 per cent. peptone (Witte or Chaputeau) and is rendered distinctly alkaline with sodium hydroxid. The medium is then clarified as usual with white of egg. It is then sterilized in the steam sterilizer in the ordinary manner. Gelatin of from 8 to 30 per cent. containing 1 to 5 per cent. peptone gives almost equally good results.

Since the coloring matter is formed only in the presence of an abundance of oxygen, cultures are made in large Fernbach flasks (antitoxin flasks) having a diameter of 20 to 25<sup>cm</sup> at the bottom, the amount of gelatin added being sufficient to give a depth of about 1<sup>cm</sup> (about 200<sup>cc</sup>). The rapid production of as large quantities of pigment as possible being the end in view, the culture flasks thus prepared are inoculated with 1 to 2<sup>cc</sup> of the liquefied gelatin poured from a very active culture of known purity, and are then placed in a closet protected from the light, where they are kept at a temperature of from 15° to 20°C. If the temperature rises much above 20° the production of pigment diminishes, and ceases if the culture be placed in the incubator. This is true for all culture media; the best results are obtained when the cultures are kept cool.

Fernbach flasks prepared as above begin to show at the end of 18 to 24 hours a decided green color which rapidly increases in intensity. At the end of three days the entire gelatin has assumed an intense, bright, grass-green, owing to the easy solubility of the pigment in water. Day by day the green becomes more intense and slowly darker, liquefaction also begins and with

<sup>4</sup>For diagnostic details the reader is referred to the articles already mentioned.

it a red dichroism makes its appearance; that is to say, the liquefied gelatin when viewed by reflected light is a pure grass-green; when viewed by transmitted light, red. The liquefied gelatin at this time cannot be distinguished in appearance from an alcoholic solution of chlorophyll. Like the latter it loses all its green tint by lamp, or ordinary gaslight (yellow light). That the coloring matter is, nevertheless, not chlorophyll will be seen from its absorption spectrum and from its behavior toward reagents.

The liquefaction of the gelatin starts at the center of the flask, where, owing to the slight convexity of the bottom, the layer of the medium is thinnest. The entire medium is soon completely liquefied, but still retains its intense green color. Gradually, however, the green changes to an olive tint and then fades away, as does also the dichroism, leaving a brownish-yellow turbid liquid.

In order to test the green coloring matter with reagents or to examine it spectroscopically, a perfectly clear solution must be obtained. This is effected by filtering the culture medium through one of the bacterial filters mentioned above. When liquefaction has not yet taken place, sufficient water is added to permit its passage through the filter tubes. When liquefaction is advanced, filtration is at once resorted to. The first runnings are rejected owing to changes in color, the result of chemical action in the pores of the filter.

The green non-dichroic solutions from young cultures give no absorption band in the neighborhood of the D line, or only the trace of one. The addition of a little acid produces a slight dichroism and a faint band appears. Acids added to any of the green dichroic solutions produce a red when in excess. Ammonium hydroxid gives also a somewhat similar color. Fixed alkalies destroy the dichroism and yield a fine clear green soon fading away. Shaking with air restores the color. These green alkaline solutions resemble the green ones obtained from young cultures and from potatoes in that they do not give an absorption band near D. The green pigment is insoluble in alcohol

and in the solvents enumerated above. The absorption spectra will be found represented in *figs. 10-16*.

If to the gelatin, prepared as has been described, a few grams of glucose, lactose, or similar bodies are added, instead of a green, a magnificent blue is obtained, permeating the culture medium completely and uniformly. The blue changes in a few days to a violet, then to a royal purple, and finally, as the culture grows old, the color disappears, the liquefied gelatin acquiring a brownish red color. Both the violet and the purple are dichroic, that is, are red by transmitted light. The liquefaction of the gelatin is considerably retarded by glucose, lactose, etc., as has been observed with many other species of bacteria.

The blue solution obtained by filtering young glucose-gelatin cultures reacts toward reagents in every way like the blue obtained from potatoes, that is, with acids first a violet, then a purple, and at last a red results; ammonia also produces a red; fixed alkalies give a green, soon fading and leaving a brownish-yellow solution which, when shaken with air, becomes first green, then blue. The absorption bands given by these solutions will be found in *figs. 10-16*.

Since it was found that these bands seemed to correspond in character, position, and intensity with those given by similarly colored or treated solutions of the pigment isolated from potatoes, no attempt has yet been made to isolate the coloring matter from gelatin. For the purposes of comparison, the filtered solutions were diluted with water until the colors given with reagents were approximately the same as those obtained with 2<sup>gm</sup> per liter of the purified pigment.

The spectra of these gelatin solutions need but a few words in explanation. A few of the most important have been given in order that they may be compared with the similarly treated solutions shown by *figs. 1-9*.

*Fig. 11* gives the absorption spectrum obtained with strongly dichroic green solutions. Acetic acid added to such solutions causes the change shown by *fig. 12*. *Fig. 13* represents the appearance seen when the violet solutions from cultures on

gelatin containing glucose, lactose, etc., are examined. It will be seen that the main band has suffered displacement toward the right, just as if an acid had acted slightly upon the coloring matter. Addition of acetic acid with the production of a purple-red color furnishes an absorption spectrum like that shown in *fig. 14* (compare this with *figs. 4, 5, 9*). *Fig. 15* is obtained when the violet solutions shown in *fig. 13* are treated with an excess of fixed alkali (in this case sodium hydroxid), and the mixture shaken with air until blue (compare with *fig. 8*).

The absorption curves represented on the plates are drawn to the scale of wave-lengths. The intensities are, of course, arbitrary, and are based upon a value which would permit of representing a spectrum such as that of *fig. 10*. Owing to the difficulty of judging the intensities of most of the solutions examined, particularly after long intervals of time, it is probable that the curves may not be perfectly accurate as to intensity. The positions of the bands, however, are correct within the limit of experimental error. The results given are averages from examinations of many cultures extending over a period of more than two years.

Most of the measurements have been made with a Krüss Universal Spektralapparat. It is, perhaps, needless to add that the usual precautions as to calibration, checking adjustments, etc., were observed.

*Growth on agar-agar.*—On nutrient agar prepared as usual and rendered slightly alkaline with sodium hydroxid, a more or less bluish-violet color is produced which diffuses through the upper part of the culture, but is reduced where the air cannot penetrate. In this case the reduction of the coloring matter has been proved by experiment to be largely due to the action of the culture medium, and not to products formed by the bacillus. In young cultures the bluish tint is the strongest, in old ones the red tint.

If to the nutrient agar glucose or lactose is added, the blue is very marked, but soon becomes violet, then purple. In the case of media inoculated by streak the change from blue to

violet starts near the inoculation streak and proceeds outward. This seems to give evidence of the formation by the organism of an acid or an acid acting substance. Such a hypothesis is supported by the fact that the red is not produced if calcium carbonate is suspended in the medium before it solidifies, thus furnishing a substance which will unite it with and neutralize any acid as soon as formed.

The comportment of the blue from agar toward reagents is identical with that of the blue from potatoes, and with that from gelatin containing glucose, etc. The absorption spectra of the different colored solutions from agar seem to correspond to those of like color obtained from other media, hence it has not been deemed necessary again to reproduce them.

*Generalizations.*—It will be noticed that potatoes, glucosed-gelatin, agar (especially if glucosed), etc., all yield a blue becoming violet, then more or less purple. Without taking more space to enumerate experiments, it can be stated that no case has yet been found where a blue has resulted in the absence of a sugar or similarly acting compound.

Agar-agar is probably closely related to the starches, and has been shown by Bauer<sup>5</sup> to contain a compound which is doubtless a sugar and, in all probability, to give rise to the formation of others through the action of acids or alkalies. Hence we have here the necessary substances to produce the blue pigment.

The violet colors on different media seem to be due to the action of an acid or acid acting substance. As the culture grows older there is more of this substance formed, and the violet changes to purple or to a red. This hypothesis is based upon the changes in position and character of the absorption bands; on the comportment of agar cultures containing calcium carbonate; and on the fact that the addition of acids to the blue solutions produce changes in color and in absorption spectra similar to those noticed in the aging of cultures. This acid may

<sup>5</sup>Journ. f. prak. Chem. II. 30:367. 1884. See also Lippmann, Chemie der Zuckerarten. Braunschweig. 1895.



possibly be acetic acid, since it has been possible to isolate alcohol and acetic acid from some cultures, yet this is by no means certain, since it is not clear whether these compounds existed preformed, or were the result of changes brought about by the analytical methods employed.

In the light of much experimental evidence extending over a considerable period of time, there seems to be no other hypothesis tenable than that all the different colors produced are simple derivatives of one and the same substance, that is to say, the organism does not produce different pigments on different media, as was first supposed, and as has been stated of many other chromogens.

The reactions and properties of this pigment do not correspond to those given by other investigators who have worked upon coloring matters of similar colors. The greens cannot be chlorophyll, nor can the blues be any one of the many cyanins which have been described. The pigment most closely resembles the coloring matters which have been isolated from lichens and from fungi, yet this resemblance is but slight.

The writers hope in a future communication to be able to announce something definite as to the nature of the pigment, and whether it belongs in reality, as now seems to be the case, to a new class of coloring matters heretofore unreported among the pigments of chromogenic bacteria.

One of the chief difficulties is to obtain sufficient purified pigment, for although its tinctorial power is very great there is but little of the material formed in each culture, and much of this is necessarily lost in the processes of purification. The problem becomes, therefore, in the first place, one of time, and cultivation of the organism on a large scale.

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## NOTES ON THE DIVISION OF THE CELL AND NUCLEUS IN LIVERWORTS.

By J. M. VAN HOOK.

(WITH PLATE XXIII)

IN the following pages are presented the results of some observations on the division of the cell and nucleus in the spore mother cell of *Anthoceros*, and in the vegetative cells of the gametophyte of *Marchantia*.

But little has been done in the cytology of the Hepaticae, aside from a study of the development of spores. Several papers were written by Farmer ('94-'95), whose detailed work has dealt, for the most part, with the cytology of reproductive cells. Along this same line Davis ('99) has worked out spore formation in *Anthoceros*, and his article upon "The spore mother cell of *Anthoceros*" strongly suggested the desirability of extending the study.

My observations on *Anthoceros* confirm his statement concerning the absence of centrospheres, but in regard to the disappearance of the connecting fibers and the formation of the cell plate by the thickening of strands of apparently undifferentiated cytoplasm which extended between the daughter nuclei my own results differ from his.

A cell plate seems to be formed in no wise different from that found in *Marchantia*, an account of which is given below, *i. e.*, the cell plate is laid down through the instrumentality of the connecting fibers (*figs. 11, 13*). Cytoplasmic strands similar to those figured by Davis are always found between the chloroplasts (*fig. 14*), but are undoubtedly *around* the spindle; and when a section includes only these strands, or if the connecting fibers be insufficiently stained, the appearance figured by him (his *fig. 25*) is presented. The same aspect is also displayed at a later stage, as in his *fig. 26*, but I am inclined to attribute

this to the persistence of the protoplasmic strands after the disappearance of the connecting fibers.

In order to see these plates as formed by the thickening of the spindle fibers, it was necessary to cut the sections two or three  $\mu$  in thickness; for at this stage the daughter nuclei lie very close together, and the protoplasm about them is exceedingly dense. Staining was protracted two or three times the usual length of time, and the excess washed out with great care.

For my study there was placed at my disposal an abundance of excellent material of a species of *Anthoceros* collected and fixed by Professor Mottier near Naples in April 1898. On account of the large size of the cells and the greater clearness with which the finer details were brought out in the preparations, this material proved to be more favorable than that obtained from the Indiana species, in which many details were brought out with difficulty.

For the behavior of the centrosome in *Marchantia* we have only the abstract from Mottier ('98). He says: "In the vegetative cells of *Marchantia* I cannot assert with absolute certainty that a definite central body or centrosome exists in all cases, but I believe that such is the case. In some in which the kinoplasmic radiations are densely stained the dark center seems to be merely the point of union of the radiations, but if the stain be washed out so that the radiations are almost colorless, a well defined and densely stained central body is generally to be seen."

It is further stated that the centrospheres are attached to the nuclear membrane and are almost diametrically opposite by the time the chromosomes are differentiated or even earlier. Farmer's statement that the centrospheres exert a pulling strain upon the nucleus is substantiated. Frequently the nuclear membrane is drawn out by their influence into slender beaks.

For the study of karyokinesis in these vegetative cells the young and rapidly growing stalks of the archegoniophore of *Marchantia* was selected. These were fixed in the Flemming

solution as used by Mottier ('97). A number of stains were used, but the best general results were obtained by the triple method of aniline-safranin, gentian-violet, and orange G. The stains were varied both in kind and length of time of use, in accordance with the structures or parts of the cell which were to be brought out.

The cells from the more rapidly growing stalk of the archegoniophore are in general cubical, and a longitudinal section of the stalk will almost invariably result in meridional sections of the spindles. The resting nuclei are round and contain large conspicuous nucleoli. The nucleolus seems to lie in the cavity or vacuole. A careful examination reveals also a very delicate network of linin threads. These are clearly seen only in successful preparations when sufficiently magnified.

In the granular cytoplasm becoming vacuolate in older cells are situated many oval or rounded chloroplasts, together with metaplasmic inclusions which stain variously. The chloroplasts and metaplasmic inclusions generally collect about the poles of the nuclear spindle, thus obscuring the finer details and rendering observation difficult.

The first noticeable evidence of nuclear division is the pulling out of the nuclear membrane, generally in the direction of the long axis of the cell, into two points which are opposite each other and around which the chloroplasts tend to collect. A careful examination reveals a minute body at each point from which extend conspicuous radiations. These bodies with their radiations are the centrospheres. They seem undoubtedly to exert a great attractive force from the manner in which certain of the cell contents are drawn to them (*figs. 2, 4*). The way in which the nuclear membrane is often drawn out into very long points would seem to indicate further that the centrosphere exerts a pulling force upon it. The exact time of the appearance of the centrosome cannot be stated with certainty, because in the absence of the radiations it is impossible to distinguish the centrosome from other bodies appearing quite like it. From what is known of their behavior in other plants it is reasonable to

suppose that they appear before the radiations, for in fact centrosomes can be recognized during the reconstruction of the daughter nucleus after the radiations have disappeared (*fig. 11*). The dense center of the centrosphere, or the centrosome, is best seen previous to the appearance of the mature spindle as a dark round body at the center of many distinct radiations. There is no reason why it should not appear as a temporary body arising from some specialized cytoplasm, as kinoplasm.

As the nucleus increases in length, the radiations become exceedingly distinct and extend farther over the nuclear membrane (*fig. 2*). They also penetrate the nuclear membrane, for any section of the nucleus at this stage will show them in its interior. Before they meet at the middle from the two centrosomes to form the spindle, the linin threads have become more conspicuous with thickened or knotty portions appearing as chromatin (*fig. 3*). This forms a network filling the large central part of the nucleus, but does not seem to extend into the pointed ends. In no case was a continuous band or spirem observed, but a framework of linin at whose crossings or thickened parts chromatin gathers. These increase rapidly in size, separate by the dissolution of the intervening linin, and form a number of chromosomes. The number seems to vary. In one instance I counted five, in another eight. These are irregularly scattered within the nuclear cavity (*fig. 4*). They are often in the form of irregular masses, but sometimes in the shape of straight or bent rods varying in size (*fig. 5*). When arranged in the nuclear plate, however, they are generally U-shaped, and each is seen to be split longitudinally.

The spindle fibers have now extended to the region of the equator, the nuclear membrane disappears, and the spindle is formed. At its center the chromosomes gather, arranging themselves at the circumference of the equatorial plane. The spindle elongates very rapidly. It is pointed, with the centrospheres yet visible at either pole. In many cases it assumes a double curve like an elongated letter S (*fig. 8*). In almost every instance at this stage this curve may be observed. Even those that seem

straight may owe their appearance to the manner in which they have been sectioned; and such is undoubtedly the case from the position of the poles and the difficulty in making an exact meridional section. This shape seems to be due to the rapid lengthening of the spindle threads as soon as they have come together at the center. Spindles are often curved, or rather it happens that the poles do not have a common axis. This is observed in their formation, however, and before the centrosomes have taken diametrically opposed positions. The daughter segments separate and pass rapidly toward the poles, along the spindle fibers, which have now become straight. Here they crowd so closely together that they lose their individuality and appear as a broken mass of chromatin, which soon becomes enveloped in a new membrane forming the daughter nuclei (*fig. 9*).

The centrosomes become invisible or perhaps dissolve as soon as the polar radiations have disappeared. The connecting fibers now thicken in the equatorial region, the first indication of a cell plate (*figs. 9, 10*). This thickening begins at the center of the equatorial plane and gradually extends to its circumference, apparently pushing the fibers farther apart toward the cell membrane, until the spindle becomes very much bulged or rounded.

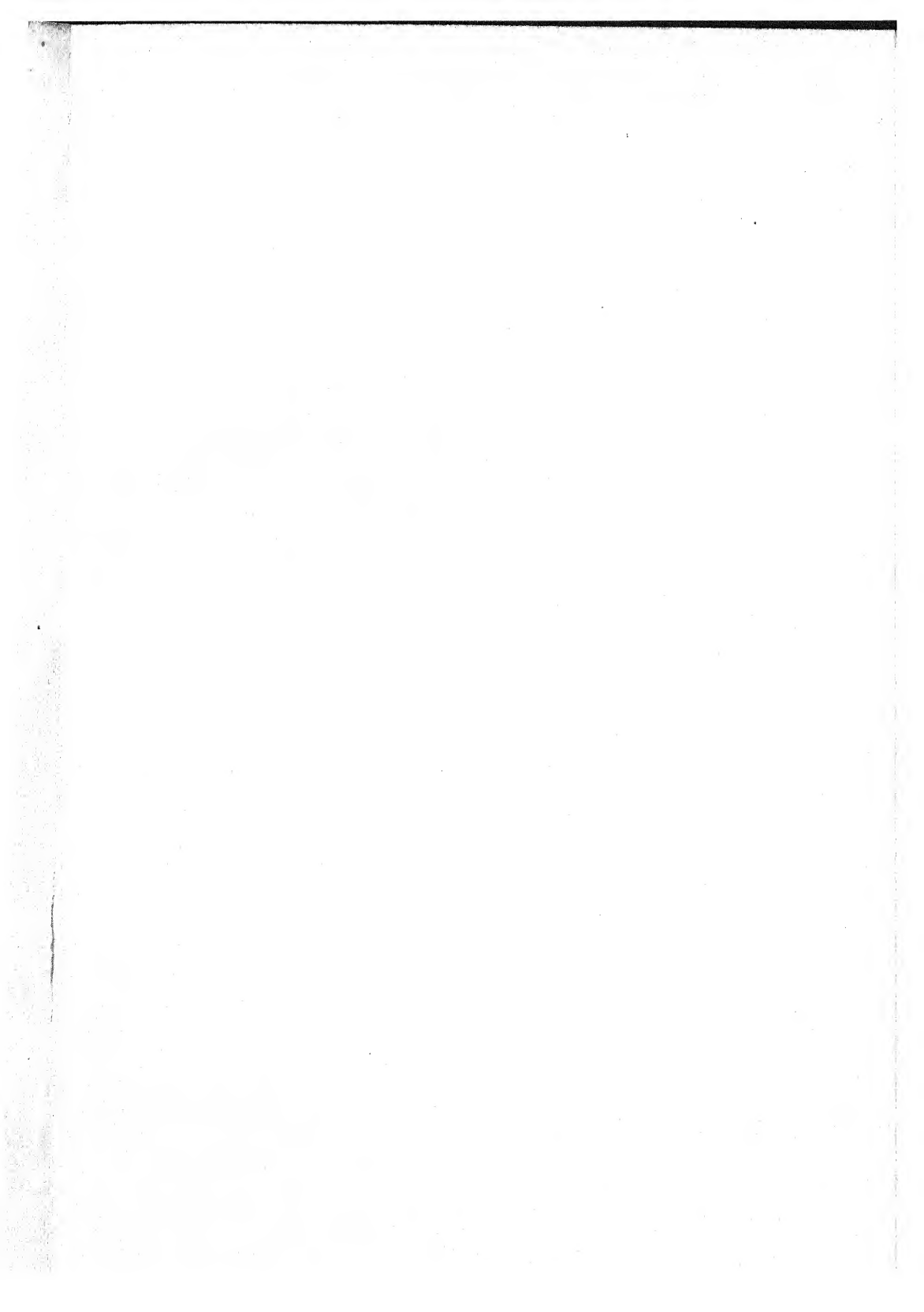
Concomitant with the bulging of the spindle, its connecting fibers begin to shorten and to dissolve at the poles (*fig. 11*). As they disappear toward the cell plate, which finally reaches the membrane, dividing the old cell completely, the daughter nuclei follow, and come to lie very near each other (*fig. 12*).

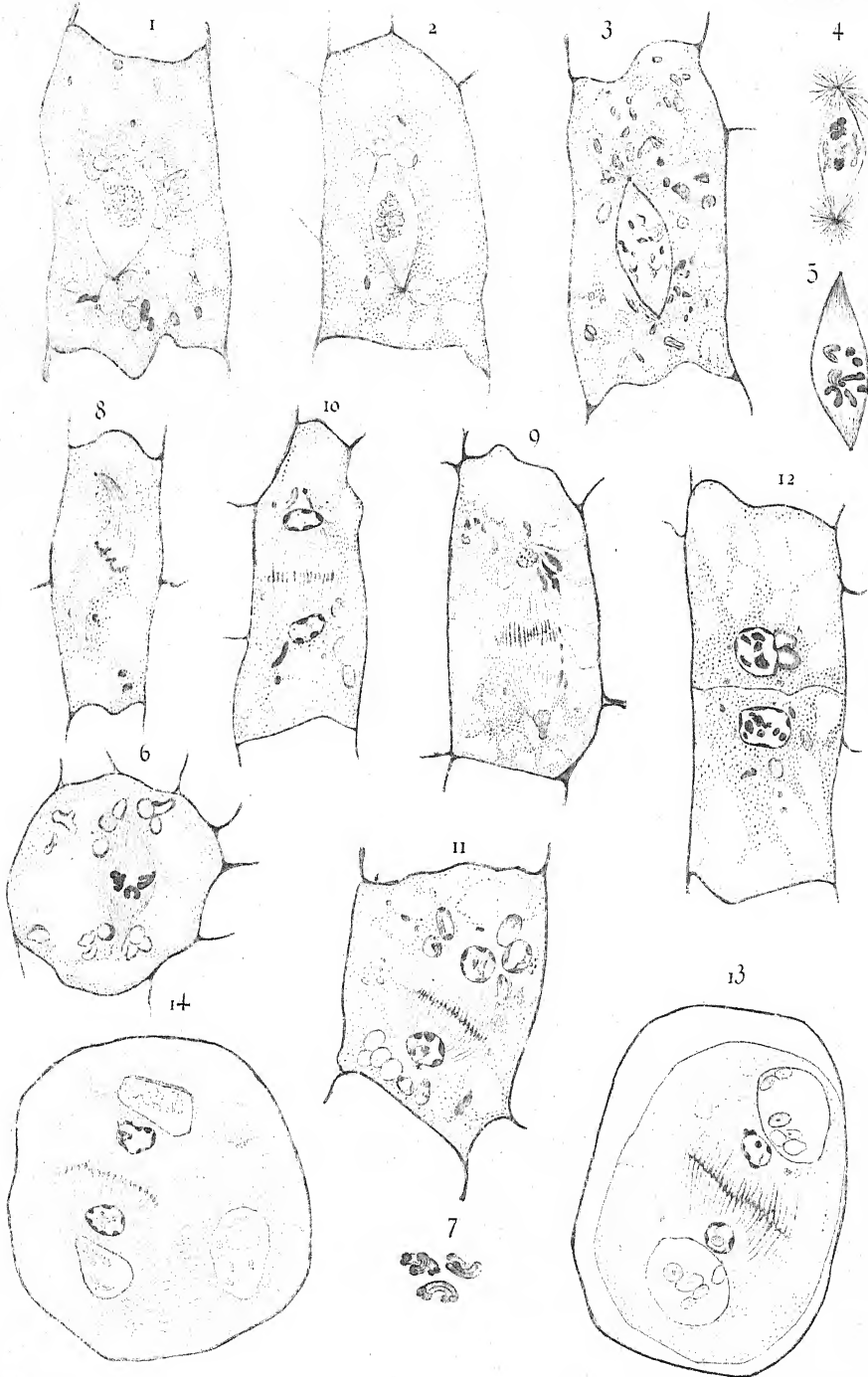
Thus the process of cell plate formation is almost identical with what I find in *Anthoceros*. It corresponds also with that described by Mottier ('97) for *Lilium*.

ANDERSON, INDIANA.

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## EXPLANATION OF PLATE XXIII.

The figures were sketched with the aid of an Abbé camera lucida; *figs.* 1, 2, 9, 10, 11, 12, 13, 14, with a Bausch and Lomb  $\frac{1}{2}$  oil immersion objective; the others with a Zeiss 2<sup>mm</sup> oil immersion and compensating ocular 12.

All preparations were fixed in the Flemming solution used by Mottier ('97), and *figs.* 11, 13, 14 were from sections 2  $\mu$  thick; the others, 5  $\mu$ . All were stained on the slide with Flemming's triple stain of safranin, gentian-violet, and orange G.

FIGS. 1-12, *Marchantia*; 13-14, *Anthoceros*.

FIG. 1. Nuclear figure from archegoniophore of *Marchantia*; the centrospheres are visible.

FIG. 2. The nuclear membrane is drawn out into points, and chloroplasts, with other bodies, are collected about the centrospheres.

FIG. 3. Nucleus previous to formation of the spindle; chromatin masses apparent in the linin net.

FIG. 4. Later stage in which the chromosomes are differentiated.

FIG. 5. Chromosomes collecting in equatorial region.

FIG. 6. Spindle stage with chromosomes arranged in equatorial plane; each chromosome is seen to be split longitudinally.

FIG. 7. Same, more highly magnified.

FIG. 8. A curved spindle.

FIG. 9. Formation of daughter nuclei.

FIG. 10. First appearance of nuclear plate.

FIG. 11. Bulged spindle, plate building toward the cell walls.

FIG. 12. Nuclear plate completed.

FIG. 13. Spore mother cell of *Anthoceros*, showing two of the nuclei only, and the nuclear plate in process of formation.

FIG. 14. Spore mother cell of *Anthoceros*, showing two of the nuclei with connecting fibers in which a cell plate is forming; cytoplasmic strands extend also between the chloroplasts and nuclei.

## BRIEFER ARTICLES.

### NEW SPECIES OF TRIMMATOSTROMA.

(WITH THREE TEXT FIGURES)

IN the summer of 1898 I had my attention called to the pathological condition of the balsams in many parts of the province of Ontario. The disease, however, had not as yet done much damage, a dead branch

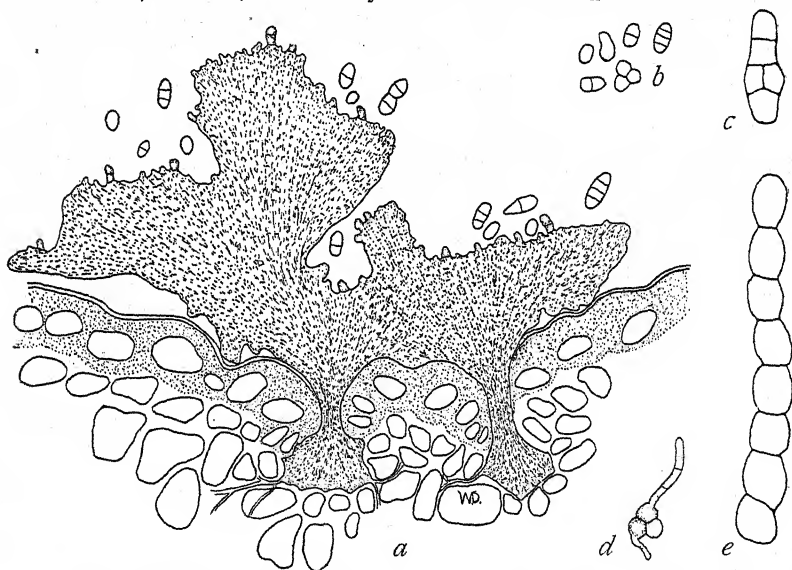


FIG. 1. *Trimmatostroma abietina*: a, cross section of a diseased leaf of *Abies balsamea*, showing the sporodochium (heavily shaded portion) and a number of loose conidia ( $\times 450$ ); b, a group of conidia of various forms ( $\times 450$ ); c, a single conidium ( $\times 1060$ ); d, germinating conidium after thirty-six hours in hanging drop of water ( $\times 450$ ); e, chain of unicellular conidia ( $\times 1060$ ).

here and there being the only warning sign of what later proved itself to be a disastrous outbreak. The woods at that time presented a peculiar mottled appearance. It was thought at the time that in this case we had to deal with a fungus form of some kind, but all efforts to collect material having it in fructification proved futile. Sections of

the diseased leaves showed the presence of a much branched mycelium made up of hyphæ which were both intercellular and intracellular. Owing to an interruption of several months, caused by the pressure of classroom work and laboratory duties, no further observations were made until the following summer (1899). The progress of destruction during this interval was far beyond expectation. Trees which but nine months before showed only a dead branch or two had now entirely succumbed, and the disease was spreading rapidly, many new points of infection being noticed. Any doubts as to the full malignancy of the disease which may have existed when it first made its appearance were now dispelled, and it was but too evident that the injury to the balsam forests would be great. The diseased leaves at this time showed the presence of numerous black warty tubercles which proved to be the fruiting masses (sporodochia). A quantity of material was collected and brought to the laboratory for further examination and study. Pure cultures were made on many different media in tubes and plates, and great difficulty was experienced in obtaining a normal development and in many cases any growth at all. Blood serum (Loeffler's mixture for diphtheria cultures, no. 8), potato agar, potato, beef agar (acid, neutral, and alkaline), and nutrient gelatin, were all tried and found serviceable as indicated in the order mentioned. Hanging drop cultures were made from the plate cultures which proved pure. In many cases the spores refused to germinate in water. Those which germinated at all did so within thirty-six hours and the majority within twenty-four, a single germ tube being put out from each spore, or in some of the multicellular forms one from each cell (fig. 1,  $\alpha$ ). From observations which I have made upon cultures and sections of imbedded material, I would characterize the form as follows:

*Trimmatostroma abietina*, n. sp.—Mycelium perennial, intercellular or intracellular, hibernating during the first winter in the tissues of

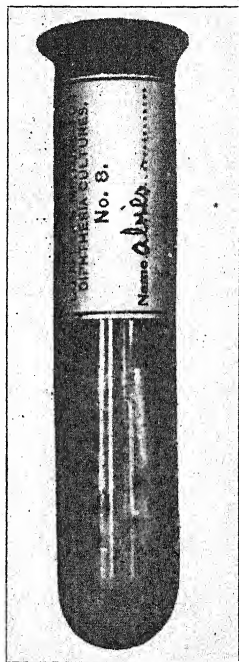


FIG. 2. A culture tube showing the growth of *Trimmatostroma abietina* upon blood serum.

the host, the following spring giving rise to conidiophores which issue from the tissues of the leaves and branches in dense fascicles forming a diffuse sporodochium. The conidiophores nearly hyaline or tinged olive brown,  $4.5\mu \times 20-30\mu$ , septate, sparsely branched, bearing the

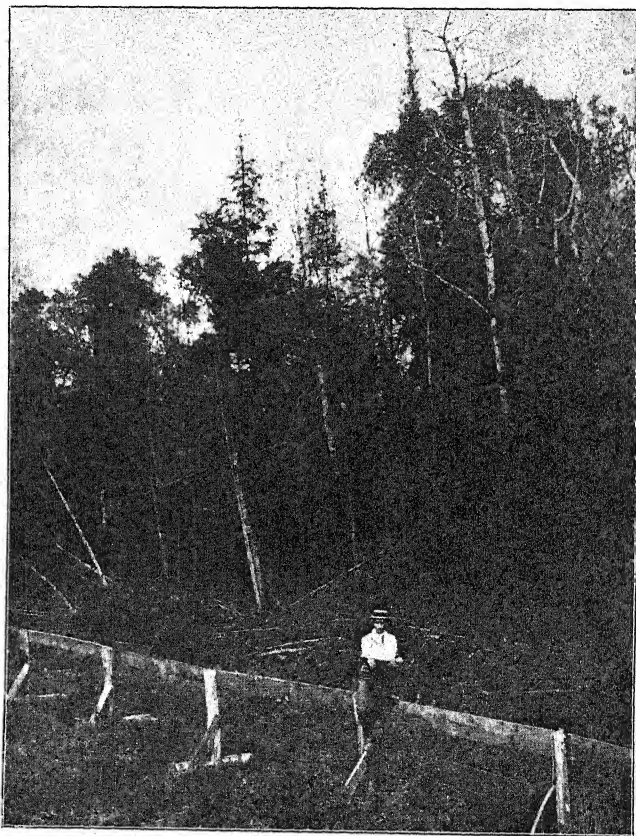


FIG. 3. Forest scene in Ontario, near Guelph, showing the appearance of a diseased area.

conidia terminally. Conidia in chains of many different kinds, all of a dark brown olivaceous color, with epispore slightly roughened, the majority oblong or spherical, usually straight, a few inequilateral; some continuous, spherical,  $5\mu$  in diameter; others septate, 2 to

5-celled, 5 to  $6\mu \times 8-16\mu$ , not constricted at the septa; and a few of the muriform type,  $5\mu \times 10\mu$ .

It will readily be seen from these characters that the form belongs to the Hyphomycetes, and in this group to the "series" Tuberculariæ dermatiæ, "section" Phragmosporæ. Further than this its identification presents many difficulties. While a large majority of the conidia are horizontally septate, and hence must be classed under Phragmosporæ, a small number have a longitudinal septum, but owing to the fact that they are borne in chains the fungus must belong to the genus Trimmatostroma. A careful examination of the literature of the subject fails to show any reference to anything of a similar form upon any coniferous tree.

Although, so far, the only hosts upon which this species has been found are *Abies alba* and *Abies balsamea*, it is very probable that the spruces (*Picea*) are not immune. Further search will be made in the hope of discovering a more mature stage of this form.

I wish to acknowledge my indebtedness to Mr. B. T. Galloway and Miss Patterson, of Washington, D. C., for having kindly assisted me in the examination of material and reference literature.—M. W. DOHERTY, *Agricultural College, Guelph, Ontario*.

## THE INTERNATIONAL BOTANICAL CONGRESS.

[Through the courtesy of our associate, Professor L. Guignard, we have received from the general secretary, M. E. Perrot, the following account of the recent meeting in Paris of the International Botanical Congress, whose sessions were held during the first ten days of October. The president of the Congress was M. le Dr. de Seynes, a former president of the Botanical Society of France; while the different sessions were presided over by MM. Drake del Castillo, Dutailly, Flahault, Mussat, and Rouy. Among the foreigners present, we note the names of Borzi, Burnat, Britton (N. L.), Chodat, Czapek, Dyer (Th.), Errera, Filarsky, Gallardo, Gamble, Greshoff, Istvanffi, Koltz, Magnus, Maiden, Micheli, Niederlein, Pfitzer, and others.—Eds.]

### I. SUBJECTS INTRODUCED FOR DISCUSSION BY VARIOUS MEMBERS.

*Methods of facilitating popular instruction concerning mushrooms.*—The introductory address was given by M. Rolland, who was followed by several members in the presentation of opinions. It was voted finally that instruction in mycology should be given down to the primary school, beginning with the recognition of poisonous species, and especially species of *Amanita* and *Volvaria*, the eating of which is

almost always followed by death. It was further stated that the illustrations of mushrooms intended for instruction should always be made under the direction of professional mycologists.

*Unification of methods employed in the determination of molds and yeasts.*—To make comparable the diagnoses of these organisms, MM. Lutz and Guéguen presented a report which proposed that investigators should substitute artificial culture media of definite and constant composition for natural culture media.

*Adoption of an international unit of micrometric measurement.*—After an address by M. Mussat, the Congress urged all botanists to use the micrometric  $\mu$ .

*Periodicity of international congresses.*—It was decided that hereafter international congresses should be held every five years and at different places. The Congress of 1905 was appointed for Vienna, with Professors Wiesner and von Wettstein in charge of the organization.

*Nomenclature.*—The Congress declared itself incompetent to revise the laws of nomenclature, but it arranged for conference upon the subject among the principal botanical societies and establishments of different countries. A representative committee will be appointed to elaborate a plan to be discussed at the Congress of 1905. M. John Briquet, curator of the Delessert Herbarium, was asked to serve as a channel of communication among taxonomists interested in the solution of the question of nomenclature, and to centralize all the correspondence.

*Phytogeographic nomenclature.*—In view of the rapid extension of phytogeography, and at the suggestion of M. Flahault, the Congress voted that all "geobotanists" should be urged to come to an agreement in reference to the terminology of the general facts of phytogeography; and to establish in the principal languages the precise synonymy of the terms recommended for use by travelers and geographers.

*Establishment of an international periodical for the publication of new botanical names.*—The subject was presented by M. Hua, who showed the advantages of some authoritative list of new names, both in avoiding the multiplication of synonyms and in simplifying publication. The Congress authorized M. Hua to devise a plan for realizing this very difficult project.

## II. THE PRINCIPAL SCIENTIFIC PAPERS.

About thirty original contributions were presented by their authors, and gave rise to more or less discussion. The papers were as follows: M. BONDIER on the influence of the soil and vegetation on the development of mushrooms; M. DANGEARD on the present status of knowledge concerning reproduction among the higher mushrooms; M. R. MAIRE on the behavior of the nucleus among mushrooms; DR. C. B. FLOWRIGHT and PROFESSOR MAGNUS on the biology of the Uredineæ; MM. CHODAT and RADAIS on the pure culture of algæ; M. CHODAT on the reactions of the nucleus to parasitism and symbiosis.

The flora of central Africa was discussed by MM. DE WILDEMAN, HUA, and A. CHEVALIER, and contrasted with that of the Amazon region, as described by M. HUBER. M. E. J. CAMUS discussed the flora of Morocco, and Dr. N. L. BRITTON gave new and interesting facts concerning the flora of the Klondike.

The physiological and anatomical papers were by MM. HOCHREUTNER, GERBER, and GIDON; while M. MARTEL presented studies on Cruciflorae, M. BEILLE on Disciflorae, M. DUTAILLY on Geum, and M. CLOS on bracts, sepals, petals, stipules, etc.

M. ANGEL GALLARDO spoke highly of the employment of the statistical method in the study of variation; while M. HUGO DE VRIES spoke upon the general subject of variation in the plant kingdom. M. PH. DE VILMORIN described a curious case of selection in *Anthriscus sylvestris*; MM. LÉVEILLE and HY spoke of hybrids; M. KRASAN treated of varieties, races, and forms; and M. GILLOT pointed out modifications which appear in local floras.

A discussion concerning the exchange of material among herbaria was participated in by MM. MOULLEFARIN, DRAKE DEL CASTILLO, and FLAHAULT.

The Congress varied the scientific conferences with visits to several collections and plantations.

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NOTES ON THE FLORA OF THE BANKS AND SOUNDS  
AT BEAUFORT, N. C.

THE coast of North Carolina near Beaufort, like most of our Atlantic coast from Long Island southward, is bordered by a line of narrow, sandy islands, or "banks," separated from the mainland by shallow sounds from one to five miles wide. These banks vary in

width from a quarter of a mile to a mile and a half, and in height from nearly sea level, in the case of certain salt marshes, to perhaps thirty feet in the case of some of the highest of the wind-blown dunes. The conditions prevailing on the higher parts of these banks are quite peculiar, for though the shifting sandy soil holds very little water, the air is very damp, since during the growing season the prevailing wind is a strong sea breeze saturated with moisture from the warm water of the Gulf Stream. Thus is to be accounted for, perhaps, the frequent occurrence here of many plants not usually found in so dry a soil, and the striking abundance of epiphytic lichens, liverworts, mosses, and ferns.

In the following pages are noted some of the interesting features of the flora of Bogue Bank and Shackleford Bank, observed during a three weeks' stay at the U. S. Fish Commission Laboratory at Beaufort, in June 1899.

The effect of physiographic agencies on the two banks above mentioned is at the present time quite different. Bogue Bank, at the point studied, seems to have reached a stage of development where it is comparatively stable. It has an outer border, or sea wall as it were, of high dunes covered with *Andropogon maritimus*, and firmly bound by its long tough rhizomes into barriers very resistant to the destructive advances of both the sea and the wind. On these dunes, growing with the *Andropogon* and within easy reach of the spray, are found scattered scrubby specimens of *Juniperus Virginiana* and large patches of *Ilex Cassine*, both overrun with *Ampelopsis quinquefolia*, *Rhus Toxicodendron*, *Smilax Bona-nox*, and *Melothria pendula*. Scattered among them are found *Chenopodium Botrys*, *Diodia teres*, *Oenothera humifusa*, and *Solidago sempervirens*.

As we go inward and downward from the outer crest of the dunes the forms mentioned give way to, or become mixed with, a very large number of other forms, making a flora much like that immediately to be described for Shackleford Bank.

On the latter bank, instead of the outer border of comparatively stable dunes, we find a shifting waste of wind-blown sand, stretching in a quarter of a mile or more from the water's edge, and reaching its greatest height at its inner border, where it is encroaching upon and rapidly burying what remains of the forest that once covered most of the bank.

For a hundred yards in from the water the only plants seen are the



everpresent *Euphorbia polygonifolia* and small tufts of *Andropogon*. At the inner edge of this shifting sand plain its surface is often ten or twelve feet above the old forest floor upon which it is advancing. It ends abruptly in a steep slope down which the sand rolls after being blown along the surface of the plain.

From the foot of this slope to the inner or sound side of the bank, a distance of nearly a mile, the surface is rolling and well covered by a thick scrubby forest made up chiefly of *Quercus virens*, *Q. aquatica*, and *Q. nigra*, with *Ilex opaca*, *Morus rubra*, *Persea Carolinensis*, *Carpinus Caroliniana*, *Juniperus Virginiana*, and *Pinus Taeda*. The highest of these trees near the middle of the bank reach a height of twenty-five or thirty feet, some of the pines perhaps exceeding this limit in the wider parts of the island.

The shrubby undergrowth between these trees is made up chiefly of *Myrica Gale*, *Ilex glabra*, *Ilex Cassine*. The latter is the most abundant of all, and near the outer border of the forest often nearly equals the trees in height. The number of species in the undergrowth becomes larger as we go in from the advancing sand plain, and soon includes many herbs, the covering of vegetation being very dense from the middle of the bank inward.

The distribution of species within this area is dependent largely upon the level of the surface. *Pinus*, *Juniperus*, and *Morus* occur chiefly upon the elevations, while *Ilex opaca*, *Carpinus*, and *Persea*, with the three species of *Quercus* noted above, occupy the hollows. These trees and the shrubby undergrowth are everywhere overgrown with dense tangles of *Berchemia volubilis*, *Vitis rotundifolia*, *Rhus Toxicodendron*, *Ampelopsis quinquefolia*, *Smilax Bona-nox*, *S. tamnoides*, and *S. rotundifolia*; often also with *Cissus stans*, *Gonolobus tuberosus*, and *Melothria pendula*.

Where the vegetation is densest, the trunks and branches of the trees and shrubs are covered with epiphytes. *Polypodium incanum* forms straggling clumps, usually on *Quercus virens*, but occasionally on the other oaks or even on the pine, becoming well established only when the tree is old enough to have a pretty rough bark. Species of *Frullania*, *Lioclaena*, *Lejeunia*, and *Archilejeunia*, form reddish or yellowish patches on the trunks of the trees, or in the case of one or two species, the delicate branches creep at the very bottoms of the deep grooves in the rough bark. Lichens, in great variety for so restricted an area, cover every available surface. On the oaks, pines, and

mulberries occur *Parmelia*, *Ramalina*, *Usnea*, and other fruticose and foliaceous forms, while the smooth barked hollies, *Persea*, and *Myrica* are literally covered from the trunk out to branches of only two or three years' growth with crustaceous *Placodiums*, *Buellias*, *Lecidias*, and others, in great variety of form and outline. One of the most striking species, occurring chiefly on the holly, forms brilliant blood red patches as large as the hand. A trunk blotched with this, intermingled with others of various shades of yellow, green, brown, and black, each patch with its clear, black outline, and often dotted with fruits of a different color, is a veritable mosaic of color.

In the lowest portions, near the middle of the banks, occur small pools of dirty water, bordered with sedges and grasses, and often also with stemless palmettos (*Sabal Adansonia*). In other, often well shaded, hollows dense patches of *Saururus cernuus* are found, and still other more open ones are completely covered with the gigantic *Sagittaria lancifolia*. Finally, among the shrubs and other herbs of these hollows are found three of the five pteridophytes seen on the banks. Those occurring here are *Aspidium Thelypteris*, *Onoclea sensibilis*, and *Osmunda regalis*. Of the other two, *Asplenium ebeneum* occurs on the drier shaded portions, and *Polypodium incanum*, as noted above, is an epiphyte.

At the inner edge of the encroaching sand plain the process of burying the old surface of the bank can be seen in all stages of progress. Just in from the foot of the slope, that is, on the outer border of the forest, many of the trees are seen to be dead, though the sand has not yet touched them. The junipers and yaupons, however, persist, and even when buried to the waist in the slope of drifting sand still look fresh and green at the top. As one goes seaward from this border between the shifting sand and the forest, these few green tops finally disappear, and then for one hundred and fifty yards or more one finds only the dead tops of trees of which all but the upper third, that is about six or eight feet, have been buried by the sand. Of these tree tops those nearest the existing forest are mostly denuded of bark, and then a little farther out one sees that the soft sapwood has been entirely carved away by the furious blasts of sand. Yet in many cases, a hundred feet from the edge of the sand slope, they are still capped by dense mats of *Berchemia*, *Ampelopsis*, or *Rhus*, and on the leeward side of the branches many lichens still flourish.

In the comparatively few days spent in examining these banks it

became evident that many ecological problems are here presented for solution. Interesting results ought certainly to be gained from a comparison of the distribution and anatomical structure of the forms occurring here with those of the same or similar forms on others of these banks farther north, where the atmospheric moisture conditions must, it would seem, be quite different.

In all nearly a hundred vascular plants were collected and identified, chiefly by Mr. W. C. Coker.

#### NOTE ON THE ALGÆ OF BOGUE AND CORE SOUNDS.

The nature of the bottom of the ocean and sounds near Beaufort is such as to provide few habitats of the sort most frequented by marine algae. There are no rocks whatever, except one or two stone wharves near the town, and several short breakwaters near the inlet from the ocean. Rock pebbles even are practically wanting, any as large as a pea being seldom found on the natural beaches. It will not be surprising, therefore, to find that the algal flora is comparatively poor in species, and is made up in numbers chiefly of those forms which attach themselves to shells lying on the sandy or muddy bottom.

Probably the most abundant alga in the sounds, except possibly *Ulva lactuca*, is *Hypnea musciformis*, which grows in thick snarled tufts on every available substratum. Scattered quite generally among the latter are tufts of *Dictyota dichotoma*, here near its northern limit, together with abundant specimens of *Rhabdonia tenera* and *Gracillaria confervoides*.

*Codium tomentosum* sometimes also occurs on shells in the sounds, but is much more frequent on the rocks of the breakwaters. On these same rocks we find *Dictyota* in abundance, often accompanied by the related form *Padina pavonia*. More abundant than all other algæ here, however, is *Sargassum vulgare*, which covers the inner breakwaters with thick tufts half a yard in length. On the *Sargassum* and the much less frequent *Fucus*, *Ectocarpus siliquulosus* and other *Ectocarpus* species are very abundant. Among the coarser forms, especially on the more exposed parts of the breakwater, grow *Enteromorpha* and most of the more delicate *Rhodophyceæ* that are found near Beaufort. Chief among the latter are *Dasya elegans*, *Erythrotrichia ceramicola*, *Gelidium crinale*, *Grinnellia Americana*, and *Trentepohlia virgatula*, with several species each of *Callithamnion*, *Ceramium*, and *Polysiphonia*.

On the leaves of *Zostera marina*, which abounds throughout the sounds *Melobesia pustulata* and probably other species are very common.

Altogether between twenty-five and thirty species were found, the most interesting feature of the flora being perhaps the occurrence here, at what must be nearly their northern limit, of the tropical or subtropical forms *Codium*, *Dictyota*, and *Padina*.—DUNCAN S. JOHNSON, *Johns Hopkins University*.

#### NOTES ON THE VALIDITY OF *ASPLENIUM EBENOIDES* AS A SPECIES.<sup>1</sup>

A RECENT visit, in company with Mr. Charles L. Pollard, to the somewhat famous locality for this fern at Havana, Hale county, Alabama, has led me to review what has been written upon the question of its hybridity, and to offer here a few comments, both upon its occurrence in Alabama and regarding its status as a species.

The theory of the hybridity of *Asplenium ebenoides* originated with Berkeley,<sup>2</sup> when, in publishing Scott's manuscript name in 1866, he expressed an opinion that the fern might well be a hybrid between *Asplenium ebeneum* and *Camptosorus rhizophyllus*. No considerable amount has since been written, but, considering the scattering references available, I think it may fairly be said that the weight of authority has been in support of Berkeley's proposition. Professor Coulter, in mentioning the discovery (1882) of new stations for this "suspicious species," remarked<sup>3</sup> that the "burden of testimony all seems to be in favor of that idea." Both Mr. Redfield<sup>4</sup> and Professor Eaton<sup>5</sup> regarded the fern as a probable hybrid; while the most concise favorable comment is that of Mr. George E. Davenport,<sup>6</sup> who cites it as "probably the best example of a fern hybrid that we have, the infrequency of its occurrence, the presence always of *Camptosorus* and *Asplenium ebeneum*, and the few plants found in the recorded stations, all going to favor the hypothesis of hybridization." In 1896 Professor Underwood, having visited the Havana locality, made the statements,<sup>7</sup>

<sup>1</sup> Published by permission of the Secretary of the Smithsonian Institution.

<sup>2</sup> Journ. Roy. Hort. Soc. 87. pl. 2, fig. 1. 1866.

<sup>5</sup> Ferns of N. Am. 1: 26.

<sup>3</sup> Bot. Gaz. 7: 37. 1882.

<sup>6</sup> Bot. Gaz. 19: 492 et seq. 1894.

<sup>4</sup> Proc. Acad. Nat. Sci., Phila. Dec. 1874.

<sup>7</sup> Bot. Gaz. 22: 410. 1896.

that "the display of the species at Havana clearly demonstrates that it is not a hybrid at all," that the fern appeared "to be multiplying, as many young plants were seen in the crevices," and that "this myth of hybridity may be put aside, for *Asplenium ebenoides* is as clearly defined a species as we possess in the genus *Asplenium*, and has no near relatives outside of its own genus." It seems to me that the conclusion adopted by Professor Underwood does not follow necessarily from the facts observed, and is perhaps less logical than the assumption, on the other hand, that the fern is a hybrid because always found under what may be termed "suspicious circumstances."

In the particularly rugged conglomerate gorge where *A. ebenoides* grows at Havana, it is indeed abundant; plants in all stages of growth abound, from the prothallus to the independent small plant and those ranging to the maximum of ten or eleven inches. *Asplenium platyneuron* (*A. ebenum*) is common in the near vicinity, but *Camptosorus* is not in great evidence, in fact, was not seen either by Mr. Pollard or myself (who made no especial search for it), though it had previously been found here in small quantity. Now, if it be agreed, as Professor Underwood assumes, that a test of this fern's hybridity consists in its inability to reproduce through its spores, most assuredly *A. ebenoides* may not be classed as a hybrid; for the presence of prothalli and young plants in such numbers precludes the possibility of their having arisen in any other way, such as by a wholesale crossing of the suspected parents. The chances of frequent crossing are indeed small, since the *Camptosorus* is present in such limited quantity; while the groups of young plants are too far distant from mature ones to suppose that they arose from proliferous buds; besides the prothalli would arise only from spores.

The question naturally arises, then, as to whether the fact of the fern's fertility effectually disposes of the supposition of its hybridity. May it not be a *fertile* hybrid? The only really well-authenticated instances of hybridization between species, of which I am aware, are those of *Phyllitis scolopendrium* (*Scolopendrium vulgare*) with *Ceterach officinarum*, *Polypodium vulgare elegantissimum* with *Phlebodium aureum*, and *Polystichum aculeatum* with *Polystichum angulare*. In the first case<sup>8</sup> the cross was effected by Lowe. Three fronds were sent by him to Mr. C. T. Druery, who has explained carefully<sup>9</sup> their intermediate

<sup>8</sup> DRUERY: Gardener's Chronicle Sept. 1895.

<sup>9</sup> Journ. Roy. Hort. Soc. 24: 292. 1900.

character, and in a recent letter briefly describes them as "scaleless fronds of true *Ceterach* form, but somewhat confluent at the tips, and with faced sori in pairs on the lower pinnae, but with single asplenioid sori on the upper portions." In the second case<sup>10</sup> the evidence is no less convincing, and the hybridity of the fern may be regarded as proven beyond question by anatomical studies, and the fact that the cross was several times repeated. In the third case<sup>11</sup> the cross (accomplished by Lowe) was between a cristate *angulare* and a dense form of *aculeatum*, resulting in a cruciate *aculeatum*. As to the fertility of these three hybrids: the sporangia of the first, contained in the fronds sent to Mr. Druery, were immature, so that no test of fertility could be made.<sup>12</sup> In the second case the spores have so far proven incapable of germination; but this sterility, Farmer suggests,<sup>13</sup> may be due to the circumstance of excessive vegetative development, for in the plainer fronds, reverting somewhat to the *vulgare* type, the spores are much better developed. In the third instance, I am informed by Mr. Druery, that, though producing spores freely, the plant proved barren in Mr. Lowe's hands, yet by others it has been found fertile, and a few plants have been raised which are freely fertile, and have given rise to a number of fine plants now in existence. Undoubtedly the parents of the latter hybrid are closely related, and a greater fertility might upon this account be expected; but had it proven infertile, as with the *Polypodium-Phlebodium* cross, it seems that even these two negative results might not properly be taken as criteria to justify the assumption that fern hybrids are sterile as a matter of course. To my mind the supposition of hybridity for *A. ebenoides* is not weakened by the discovery of its evident fertility. I am told by Mr. H. J. Webber that in the case of spermatophyte hybrids between distinct species the percentage of fertility is very much in excess of the common belief; the hundreds of sterile crosses here count for nought as an argument leading us to *expect* sterility, in the presence of an equal host of fertile ones. And so it may be in the case of fern hybrids. It appears entirely gratuitous to assume that there can be

<sup>10</sup>FARMER: *Annals of Botany* 11: 533 *et seq.* 1897.

<sup>11</sup>LOWE, *Fifty years fern growing*, 1896. DRUERY: *Journ. Roy. Hort. Soc.* 24: 292. 1900.

<sup>12</sup>Mr. Druery states that these fronds are still in his possession, but that the plant has probably not survived.

<sup>13</sup>FARMER: *Annals of Botany* 11: 533 *et seq.* 1897.

no such thing as fertile hybrids between fern species. If we grant the specific distinctness of *aculeatum* and *angulare*, the converse has now been proven. The fertility of certain varietal crosses seems well established, and it may be regarded as by no means improbable that, with the use of careful methods, the same may be unquestionably proven in the case of a good number of specific hybrids.

Regarding the relationship of *A. ebenoides* to its supposed parents, Professor Eaton remarked<sup>14</sup> that "while it differs from the first [*Camp-tosorus*] by its dark and shining stalk and rachis, in its free veins, and by its pinnatifid or sub-pinnate frond, it resembles it strongly in the prolonged and slender apex, and especially in the proliferous buds; and in the very respects in which it differs from this it resembles the other." These very features of resemblance appealed to Berkeley with sufficient force to induce him to advance the hybridity proposition; but Professor Underwood apparently attaches little importance to them, considering the plant closely allied to *Asplenium pinnatifidum*, in which he is supported by Professor Murrill.<sup>15</sup> The fern seems to me to exhibit conspicuously certain characters of both its supposed parents, not the least important of which is the more or less frequent anastomosing of the veinlets. This fact is easily noticeable in a good share of the specimens, as noted by Professor Murrill in commenting upon Professor Eaton's statement to the effect "veins are everywhere free," a character distinguishing all true *Asplenium*. Its propagation through proliferation at the apex, questioned by Professor Murrill, was vouched for<sup>16</sup> in cultivated plants as early as 1878 by Mr. Davenport, and lately Mr. William Palmer has collected fronds in Maryland in which the pinnae bear at their tips minute but well-formed young plants. A good proportion of the plants collected this summer at Havana by Mr. Pollard and myself bear young plants at the apex of the fronds, but I have yet to notice any of fair size and large enough to thrive if separated from the parent plant. I am inclined to believe that these young plants never come to an independent existence. If they are indeed abortive, the fact may be taken as tending to substantiate the claim for its intermediate position.

Another feature in the morphology of the plant which seems especially significant is its marked asymmetry. Even in examining the great growth at Havana it is extremely difficult to discover what would

<sup>14</sup> Ferns of N. Am. 1: 26.

<sup>15</sup> Fern Bull. 5: 1. 1897.

<sup>16</sup> Bull. Torr. Bot. Club 6: 200. 1878.

in an ordinary species be designated as a "perfect frond." Something of this tendency is occasionally seen in *Asplenium pinnatifidum*, but in *A. ebenoides* it is at once the most noticeable and constant feature. In its ordinary mature development it is as constant in this irregularity of shape as most ferns are in their symmetry; this in itself indicates an unusual phylogeny and is a strong bit of evidence supporting its claim to hybridity. A like trait has been noted<sup>17</sup> by Mr. Davenport in the case of his *Dryopteris cristata*  $\times$  *marginalis*, which, it may be added, Miss Slosson's recent experiments bid fair to prove a true hybrid.<sup>18</sup>

It may be noted further that at Havana *A. ebenoides* in its habitat resembles neither of its supposed parents, both of which require considerable humus and grow mostly upon rocks. *A. ebenoides*, however, is here strictly a mural fern, and like *A. pinnatifidum* and *Pellaea atropurpurea* grows in the narrow chinks of rock where there is a minimum of earth. It is for the most part well shaded and must derive most of its moisture from the porous conglomerate cliff, on the under side of which it grows. The cause of its abundance here is undoubtedly to be found in the especially favorable environment, and perhaps a greater fertility of spores than in the other stations. That it has not a wider distribution in the general region is no more remarkable than the limited distribution of other species, and is perhaps due to a dearth of suitable situations. But Professor Murrill's observations upon the plants found by him in six or seven different localities near Blacksburg, Virginia, show that the fern does well in light rich soil; while a recent letter from Dr. Rusby states that the plants found by him<sup>19</sup> near Newton, N. J., grew in an open sunny spot on a dry limestone ridge; so that after all its habitat is variable. Whenever *A. ebenoides* has been found elsewhere than at Havana it has usually, if not always, been a plant or two in a place, and these rarely in the same general region. What is there to account for this isolation? They might arise from wind-blown spores; but in this event is it not a singular coincidence that the fortunate spore happens on each occasion to settle between plants of *Asplenium platyneuron* and *Camptosorus*? Besides, the stations are mostly separated by considerable distances, one in Vermont, another in Connecticut, in New York, and so on. Thus, though it be a possible, we may hardly regard it as a probable means of origin. Is it not then somewhat plausible to suppose a local origin for the fern at

<sup>17</sup> Papers Pres. Bost. Meet. (1898), 10. 1899.

<sup>18</sup> Fernwort papers. (Ined.)

<sup>19</sup> Bull. Torr. Bot. Club 7: 29. 1880.



each of its few stations by means of a natural hybridization? The rarity of its occurrence is a matter of fact; and, granting the hybridity supposition, it is evident that from the resulting plant of a single cross excessive multiplication is hardly to be expected, be the spores ever so fertile. Evidently much would depend on the environment, and it is easy to assume that the Havana station has proven most favorable, the Virginia locality rather less so, and the remaining stations barely capable of supporting their foundlings. In view of anything like conclusive evidence at the present time it is obviously impossible to settle the contention; my effort has been merely to bring together the related facts known, and to suggest again the possibility or perhaps the probability of such a solution.

It is evident, as has been both affirmed and denied, that the burden of proof rests with those who claim hybridity for the fern; it remains for the sponsors for that theory to prove their contention by actual demonstration. It can be done only by painstaking artificial crossing of the supposed parents; and I am inclined to the belief that this is possible. It is easier, admittedly, to proclaim the hybridity of the fern a myth, its abundance at Havana sufficient evidence that it is a distinct species, the scattering plants mere relics of a more general northerly distribution, and all else mere speculation; but it appears to me that the invariable presence of both supposed parents, the anomalous appearance and peculiar morphology of the fern, its usual occurrence singly and in this connection its very rarity embrace a suggestion of hybridity too patent to be ignored, and of sufficient interest to warrant careful cultural experiments.—WILLIAM R. MAXON, *U. S. National Museum, Washington, D. C.*

## CURRENT LITERATURE.

### BOOK REVIEWS.

#### *Elements of paleobotany.*<sup>1</sup>

THE great needs of recent years in paleobotany have been a summary of the scattered materials and the delimitation of well-founded data from those which are more or less uncertain. A great stride forward has been taken along these lines and as a result we are in a position to speak more categorically as to plant fossils. The first part of Professor Seward's work appeared some time ago and has been reviewed in these pages.<sup>2</sup> Almost simultaneously three valuable works have recently appeared, one in English by Professor Scott,<sup>3</sup> one in German by Potonié, and the one which is the subject of this review.<sup>4</sup> The standpoint of the three works is somewhat different, Scott taking more the standpoint of the morphologist, Potonié of the stratigrapher, while Zeiller combines the botanical and geological standpoints, though giving more emphasis to the botanical side. More than any book that has yet appeared, this is a book to be used with impunity by general readers and elementary students. The first chapter treats of the various methods by which plant fossils have been preserved, then follows a chapter on classification and nomenclature. The body of the book, of course, is made up of descriptions of the various fossil forms treated in order. The cuts are simple but clear and good, and the descriptions are doubtless the shortest and clearest that are found anywhere.

The conservatism of the author is shown at many points, and the difference between established and hypothetical data is clearly brought out. As an illustration of this, Zeiller constantly distinguishes between forms based on leaves and forms based on reproductive organs, as in the ferns. There are interesting discussions of the Sphenophylleæ and the Cycadofilices, though the author does not go so far as some in erecting these forms into great groups by themselves.

At the close of the book are two chapters of extreme interest. The chapter on the succession of floras and climates is wonderfully graphic, and it is doubtful if a better summary of the known facts was ever written, certainly not in a shorter compass. The author theorizes but little from the

<sup>1</sup> This review also appears in the *Journal of Geology*.

<sup>2</sup> Bot. Gaz. 26:59. 1898.

<sup>3</sup> Bot. Gaz. 30:352. 1900.

<sup>4</sup> ZEILLER, R.: *Éléments de Paléobotanique*. 8vo. pp. 421, with 210 illustrations, Paris: Georges Carré et C. Naud. 1900.

facts presented, and such deductions as he makes in regard to climate are conservative. The last chapter will be somewhat startling to many readers, as Zeiller thinks there is very little evidence from fossil plants in favor of gradual evolution. He states that in almost every case, species, genera, families, and groups appear highly specialized and in their permanent form from the first. So-called intermediate forms like *Cheirostrobis* appear long after the forms they are supposed to connect. Genera and species that vary now have always varied, and the limits of variation now and in the past have been the same and definitely prescribed. In short, Zeiller believes that the evolution of all groups is a matter almost purely of speculation. Doubtless most botanists will fail to accept Zeiller's views as to evolution, and yet it may be well to put a brake now and then to unlimited speculation; a perusal of Zeiller's final chapter certainly compels one to do that.—H. C. COWLES.

#### Plant diseases.

IN 1882 the first edition of Robert Hartig's *Lehrbuch der Baumkrankheiten* appeared. This book met with instant favor and was at once recognized as a standard reference work for diseases of trees, especially those caused by the higher fungi. In 1889 the second edition appeared and the favorable reception accorded the first edition was repeated. The third edition has now been issued—this time, however, with a changed title.<sup>5</sup> The change of name from *Baum-* to *Pflanzenkrankheiten* would naturally lead one to expect that the discussion of the subject had been extended so as to include non-woody plants not considered in the previous editions. This is not the case, however, for practically the same plants are treated in the edition before us as in the others. The work is still confined in the main to diseases of woody plants. This is shown by the fact that of the 280 figures only eleven illustrate diseases or parasitic fungi of non-woody plants. Thirty-one pages are given to the discussion of the rusts affecting woody plants and a little over two pages to those affecting non-woody plants. Aside from about six pages given to smuts and short references under *Claviceps purpurea*, *Cystopus candidus*, *Plasmidiophora brassicae*, and the bacterial diseases of hyacinths and potato, the other notes on diseases of non-woody plants caused by fungi are only incidental. The book is divided into five main headings, viz.: (1) injuries caused by plants; (2) diseases caused by atmospheric influences; (3) diseases caused by the action of injurious substances; (4) diseases due to soil conditions; (5) wounds. With the exception of one or two paragraphs, however, consideration is given under the last four heads to woody plants only.

<sup>5</sup>HARTIG, ROBERT: *Lehrbuch der Pflanzenkrankheiten*. Für Botaniker, Forstleute, Landwirthe, und Gärtner. Dritte, völlig neu bearbeitete Auflage des Lehrbuches der Baumkrankheiten. 8vo. pp. ix + 324. figs. 280. pl. 1 (colored). Berlin: Julius Springer. 1900. M 10.

As compared with the second edition, a somewhat fuller discussion is given to diseases caused by fungi as a whole, and particularly to Ustilagineæ and Uredineæ. The injuries caused by lightning are much more extensively treated than in the previous edition, being given over fourteen pages and twenty-six figures instead of two pages and no figures. The number of figures in the book as a whole has been more than doubled, many being reproductions of photographs to show the habit of certain diseases, while others are detailed drawings made by the author. The colored plate which appeared in the first edition has been retained. In view of the great activity shown in recent years in the study and description of bacterial diseases of plants it seems peculiar to find only two pages devoted to this important subject. Practically the same introductory remarks are given as in the second edition, to the effect that bacterial diseases of plants are not and cannot be numerous in the nature of things, owing to the firm cellulose walls and acid sap characteristic of most plants. But five bacterial diseases are mentioned, two being considered as not yet proved. They are the yellow rot of the hyacinth, the wet rot of the potato, pear blight, and more doubtfully sorghum blight, and the twig gall of the olive. It seems strange that no mention is made of several other economically very important bacterial diseases which have been so carefully and fully described by European and American investigators. A very noticeable feature is the fact that except for a few citations in the introduction to general works, all the publications cited are those by the author himself. He explains this as being done to make these publications more available, since they are scattered through numerous periodicals, etc. There is no need, he believes, for a general bibliography in such a work.

The typography is good, and the figures are mostly excellent. The only typographical error noted is in the legend to *figure 123*, where the name *Cryptospora* is printed instead of *Calyptospora*. As a whole the book is likely to prove valuable, not as a general work, however, but rather as a text-book of diseases of trees.—ERNST A. BESSEY.

#### MINOR NOTICES.

HERMAN B. DORNER (Proc. Ind. Acad. Sci. 1899: 116-129) has investigated the resin ducts and strengthening cells of the leaves of six species of *Abies*, and five of *Picea*, and has discovered differences which enable him to distinguish them. The paper contains text cuts showing the varying structures.—J. M. C.

THE LAST NUMBER of the *Icones Florae Japonicae* bears the date September 1, 1900, and is no. 8 of the spermatophyte and pteridophyte series, edited by T. Makino of the Imperial University at Tōkyō. The present number contains illustrations of species of *Davallia*, *Aldrovandā*, *Stigmatodactylus*, and *Saccolabium*.—J. M. C.

THE FIFTH FASCICLE of Engler's work on the genera and families of African plants<sup>6</sup> has just appeared, containing Professor Schumann's presentation of the Sterculiaceæ. The genera are 16 in number, and the species 211, the large genus being *Hermannia* with 73 species. The appearance of these monographs is sumptuous, and the plates are beyond praise.—J. M. C.

A NOTICE of the appearance of the first fascicle of Halácsy's *Flora of Greece* was published in this journal for April last (29:290. 1900). In that notice the occasion for the work and its general character were set forth. The second fascicle<sup>7</sup> has now appeared, and completing Alsiniaceæ continues to the beginning of Crassulaceæ. The sequence is that of Bentham and Hooker.—J. M. C.

THE FIRST REPORT of the Michigan Academy of Science has appeared. Its numerous papers indicate a strong and promising organization. The botanical contributions, aside from some general papers by Professor Beal, are as follows: The flora of Michigan lakes, and Teratological forms of *Trillium grandiflorum*, besides several abstracts, by CHARLES A. DAVIS; On saprophytic fungi in the vicinity of the Agricultural College, by B. O. LONGYEAR.—J. M. C.

MR. G. E. STONE, of the Massachusetts Agricultural College, has published a list of the plants of Lake Quinsigamond, a lake near Worcester, and one of the largest in Massachusetts. The flora is evidently one of great interest, and includes some plants which are restricted in central Massachusetts to this lake and its tributaries. The summary shows 88 spermatophytes, 4 pteridophytes, 15 bryophytes, and 331 thallophytes (319 of which are algæ), making 450 species in all.—J. M. C.

ALEXANDER W. EVANS (Proc. Wash. Acad. Sci. 2:287-314. *pls.* 16-18. 1900) has just published a paper on the Hepaticæ collected in Alaska by the Harriman expedition. There were collected sixty-three species in a condition to be identified, thirty-eight of which are recorded from Alaska for the first time. There are known in Alaska at present eighty-two species of Hepaticæ, sixty-seven of which belong to the Jungermanniales. No representatives of the Ricciaceæ or of the Anthocerotales are as yet definitely known from the territory.—J. M. C.

LESTER F. WARD (Amer. Jour. Sci. 10:327-345. *pls.* 2-4. 1900) has described a number of new species belonging to his genus *Cycadeoidea*. These cycadean trunks are from the Black hills. The described species

<sup>6</sup> ENGLER, A.: Monographien afrikanischer Pflanzenfamilien und -Gattungen. V. Sterculiaceæ, bearbeitet von K. Schumann. 4to. pp. 140. *pls.* 16. Leipzig: Wm. Engelmann. 1900. *M* 30.

<sup>7</sup> HALÁCSY, E. DE.: Conspectus Floræ Græcæ. Vol. I. fasc. II, pp. 225-576. Leipzig: Wilhelm Engelmann. 1900. *M* 8.

now number twenty-nine, and all of them are represented in the collections of the Yale museum. Professor Ward ventures the opinion that we are only upon the threshold of knowledge in reference to the extinct floras of America, and he voices the hope of botanists by stating that "as we penetrate deeper into the inner structure, it is safe to predict that the results will be such as even the botanists proper cannot afford to ignore."—J. M. C.

DR. SMITH ELY JELLIFFE<sup>8</sup> has published a catalogue of the flora of Long Island. His reason for publishing a single list for an island which possesses numerous local floras "lies in the peculiar features of botanical interest that are caused by the position of the island in relation to the mainland, and the history of the growth of plant life on the Atlantic slope, as shown by the combined evidence of geology and botany on Long Island." The author makes an interesting attempt to relate the present flora to what he calls "geological floras." The following statistics of the flora are given: Thallophytes, 719; Bryophytes, 136; Pteridophytes, 41; Spermatophytes, 1342 (Gymnosperms 14, Monocotyledons 322, Dicotyledons 1006); total number of known species 2238.—J. M. C.

FASCICLES 202 and 203 of Engler and Prantl's *Die natürlichen Pflanzenfamilien* have appeared as a double fascicle, completing the first part of the first volume. It is devoted entirely to Flagellata, by G. Senn. The general structure of these somewhat problematical organisms is dealt with in a very satisfactory way. A summary of the group shows the following statistics: seven orders, twenty families, 128 genera, and about 350 species. One cannot but remark the great preponderance of monotypic genera.

The first supplement to this work has also just been issued, including the new material of the year 1897-8 for volumes II-IV. It contains not only the new genera which have been described, but also references to important additional literature. The continuation of these supplements will be of very great value.—J. M. C.

A. A. HELLER has issued a second edition of his "*Catalogue of North American Plants north of Mexico*", bearing the date of November 10, 1900. The first edition was issued in March 1898, since which time the editor states that about two thousand new plant names have been published. The second edition possesses all of the features desirable in a catalogue of this nature; the page is printed upon one side only, giving space for the insertion of new names; larger type has been used than in the previous edition, and the press work is beyond criticism. Such complete lists are absolutely essential to the taxonomist and to those interested in building up collections. The editor gives the interesting calculation that "at the present rate of activity in taxonomic botany, the year 1905 will see twenty thousand plant names to be

<sup>8</sup>The flora of Long Island. 8vo. pp. xvi + 160. Lancaster, Pa.: The New Era Printing Co. 1899. \$1.

listed and many changes in generic limits." The list of names is preceded by five pages of changes in nomenclature, which of course involves the citation of the synonyms.—J. M. C.

THE *Flora of the West Indies*, by Urban,<sup>9</sup> has reached the second fascicle of the second volume. The two previous fascicles were noticed in this journal for April last (29: 289. 1900). The new fascicle contains the conclusion of the Cyperaceæ by C. B. Clarke, followed by Urban in a long list of corrections of nomenclature and spelling of generic names in the family more in accordance with modern views. The Acanthaceæ are presented by G. Lindau, 37 genera and 90 species being recognized, the new genera being *Drejerella*, *Ancistranthus*, and *Centrilla*. *Dianthera* is merged under *Justicia*. New species of Lauraceæ and Bromeliaceæ are described by C. Mez. "New or little known Leguminosæ" is the title of a series of papers by I. Urban, the first one containing about 30 new species, the majority of which belong to *Caesalpinia* and *Galactia*. The new genera are *Hebestigma* (founded on *Robinia*? *Cubensis* HBK.) and *Rhodopis* (founded on *Erythrina planisiliqua* L.).—J. M. C.

THE PUBLICATION of a state flora does not mean very much in these days unless it devotes considerable attention to the ecological standpoint. The new state flora of Indiana<sup>10</sup> is particularly welcome because of the full ecological as well as geographical notes in connection with each species. Instead of being a mere list, valuable only to the collector, there is material of value to all botanists. In the introduction the physiographic features, climate, and soils are described briefly, and then the author discusses the plant societies. After a statement of general principles, the hydrophytes, xerophytes, and mesophytes are treated in order, especial attention being given to the xerophytes. Topics of more general economic interest come next, notably reforestation, poisonous plants, and weeds. A very complete bibliography closes the introduction, and the remainder of the book (400 pp.) gives a detailed account of the various species. Through an unfortunate oversight the reprint is not dated, nor is there any indication as to its source.—H. C. COWLES.

T. C. PALMER and F. J. KEELEY (Proc. Acad. Nat. Sci. Philad. 1900: 465-479. *pls.* 15, 16) have published concerning the structure of the diatom girdle. They have noticed that in all literature upon diatoms there is a tendency to neglect the structure of the girdle and to take for granted that it

<sup>9</sup> URBAN, IGNATIUS: *Symbolæ Antillanæ seu fundamenta floræ Indæ occidentalis*. Vol. II, fasc. 2, pp. 161-335. Berlin: Gebrüder Borntraeger. 1900. M 9.90.

<sup>10</sup> COULTER, STANLEY: A catalogue of the flowering plants and of the ferns and their allies indigenous to Indiana. Separate reprint from Report of the Indiana State Geologist, 1899, pp. 553-1074.

is everywhere the same, and that it is a typically closed hoop. Their present claim is that the closed hoop structure is unusual. They make the statement that "with some very important exceptions, the girdle is a two-ended band of silica, with the ends variously and characteristically rounded or otherwise modified, and approximated or overlapping without being joined. The position of the gap or joint is, within limits, constant in a given genus with relation to salient features of the valves. In case of each simple pair of primary girdles, the two gaps are usually at opposite points of the diatom; and in general, in the forms we have studied, the gaps are normally so situated with respect to each other as to 'lap joints.'" These claims are supported by the presentation of typical examples.—J. M. C.

#### NOTES FOR STUDENTS.

THE EFFECT of certain inorganic salts in accelerating the growth of lower plant organisms has recently been made the subject of research by N. Ōno.<sup>11</sup> He has worked with the following substances in very dilute solutions:  $\text{ZnSO}_4$ ,  $\text{FeSO}_4$ ,  $\text{NiSO}_4$ ,  $\text{CoSO}_4$ ,  $\text{CuSO}_4$ ,  $\text{HgCl}_2$ ,  $\text{LiNO}_3$ ,  $\text{NaF}$ , and  $\text{K}_2\text{AsO}_3$ . For fungi *Aspergillus niger* and *Penicillium glaucum* served as subjects; for algæ (and this is the first time this group has been brought under experimentation in this regard), *Protococcus* sp., *Chroococcum* sp., *Hormidium nitens*, and *Stigeoclonium* sp. Suitable nutrient fluids were used unmodified for the control cultures, and with addition of one of the above-named salts for the tests.

With the exception of  $\text{CuSO}_4$  and  $\text{HgCl}_2$ , all of the salts, in very dilute solution, act upon the algæ to accelerate vegetative growth and to retard the process of zoospore production. All of the salts act in this way upon the fungi studied; in this the author is in accord with Richards.<sup>12</sup> For the algæ the optimum growth is obtained by addition of a much smaller amount of the unusual salt than for the fungi. In the former case the concentration of this compound varies from  $\frac{1}{2,500,000}$  to  $\frac{1}{100,000}$  gram molecule per liter; in the latter from  $\frac{1}{4000}$  to  $\frac{1}{800}$  gram molecules per liter. Sixteen pages of tables give the results very clearly. The author has been content to determine the facts as stated above, without inquiring more deeply into their nature. He seems to make the mistake of considering his salts as remaining intact in these dilute solutions, instead of being completely dissociated, as they almost certainly are. It is very probable that (excepting in case of

<sup>11</sup> Ueber die Wachstumsbeschleunigung einiger Algen und Pilze durch chemische Reize. Jour. Coll. Sci. Imp. Univ. Tōkyō. 13: 141-186. pl. 13. 1900.

<sup>12</sup> Die Beeinflussung des Wachstums einiger Pilze durch chemische Reize. Pringsh. Jahrb. f. wiss. Bot. 30: 665. 1897.



the last two salts in our list) the accelerating stimulus is due to the cations, and if this be true it could easily have been demonstrated by the use of equimolecular solutions of different salts of the same metal. That the effect is not due to the anions we must conclude from the fact that the control solutions contains  $\text{SO}_4$ -ions from  $\text{MgSO}_4$ , and  $\text{NO}_3$ -ions from  $\text{NH}_4\text{NO}_3$  and  $\text{KNO}_3$ . It may be profitable to call attention to the fact that where  $\text{NaF}$  and  $\text{K}_2\text{AsO}_3$  are used the stimulus to growth must be due not to the cations but to the anions. However, the material at hand is far too meager for any general conclusions along this line.

If our author has seemed somewhat easily satisfied as to the chemical nature of the stimulus with which he is dealing, the omission is palliated by a decided advance which he has made in another line. Heretofore experiments upon acceleration and retardation of growth have gone no further than to determine the relative increase in somatic material. Now, since of the materials taken into the plant body some are used to increase its mass and others go out again more or less completely oxidized, thus contributing energy rather than matter to the organism, it becomes essential to determine whether or not accelerated growth means also a relative increase in the amount of waste products. Of course in the algæ these products of katabolism take the form mainly of  $\text{CO}_2$ , and, owing to the photosynthetic process, its amount cannot be easily determined. But in fungi we have no such disturbing factor. Much of the waste from these fungi comes off in the partially oxidized form of oxalic acid. The relative amounts of this excreted in the different cases was determined by titration and compared with the amount of sugar taken from the fluid by the growing plants. Relatively much more acid was given off in the normal cultures than in those containing  $\text{ZnSO}_4$ ,  $\text{CuSO}_4$ ,  $\text{CoSO}_4$ ,  $\text{HgCl}_2$ , and  $\text{NaF}$ . For example, with  $\text{ZnSO}_4$ , in twenty-five days *Aspergillus niger* gave off per gram of its own weight 0.443<sup>gm</sup> of acid. Without  $\text{ZnSO}_4$  the amount given off was 2.245<sup>gm</sup> per gram of its own weight.  $\text{NiSO}_4$  was the only exception; it acted in the opposite way. What are called "economic coefficients" are obtained by dividing the amount of sugar used by the amount of substance produced in the plant body. This coefficient is much greater in the control cultures than in the others. They average about as one to two. If the weight of the fungus and that of the sugar absorbed could have been reduced to calories, and then this coefficient found as above, it will be seen that it would have been the reciprocal of what, in mechanics, would be termed the efficiency. Thus the result above enunciated seems to show at least that the efficiency of the fungus, as a machine for storing up material, increases with the addition of traces of these poisons. This seems to open up a new method for obtaining some quantitative measurements of the metabolic process. But since the author did not have it in mind his data are not complete enough for this purpose.

One other point is made in the paper. Loew has shown<sup>23</sup> that the spore-producing function of fungi is much more susceptible to the action of poisons than is the vegetative. Thus it should be possible to obtain a concentration of the poison such that it would hinder or stop spore production and still not effect, or effect in a minimum degree, the vegetative growth. But under these conditions the energy normally directed to spore production would probably be turned towards growth. Thus we would have an acceleration in growth due to the retardation in reproduction. This, the author concludes, is probably the case in his researches.—BURTON EDWARD LIVINGSTON.

ITEMS OF TAXONOMIC INTEREST are as follows: W. N. SUKSDORF (Bot. Monatsschrift 18:97-99, 132-134. 1900), in continuing his descriptions of Washington plants, has published new varieties in *Viburnum*, *Valerianella*, *Maidia*, *Artemisia*, *Troximon*, *Dodecatheon*, *Phlox*, and *Gilia* (3), and new species of *Dodecatheon*, *Gilia*, and *Amsinckia* (4).—In continuing his studies on the fungus flora of South America, DR. H. REHM (Hedwigia 39:209-224. 1900) has reached the Discomycetes, in which he describes *Physmatomyces* (Bulgariaceæ) and *Psorotheciopsis* (Mollisieæ) as new genera.—PASCAL CONTI (Mém. Herb. Boiss. no. 18:1-86. 1900) has published a revision of the species of *Matthiola*, recognizing 32 species, 5 of which are described as new.—MARCUS E. JONES (Zoe 5:41-53. 1900) has published the ninth of his "Contributions to Western Botany," which contains many critical notes, some new forms of *Astragalus*, and a very interesting ecological sketch of the Great Salt Lake desert.—WILLIAM F. WRIGHT (*ibid.* 53-58) has published seven new species and two new varieties of *Galium*, chiefly from California.—E. J. DURAND (Bull. Torr. Bot. Club 27:463-495. pls. 27-32. 1900) has published a classification of the fleshy Pezizineæ, segregating them into four families on the basis of the structure of the sterile layers of the cup.—E. J. HILL (*ibid.* 496-505) has published an interesting account of *Celtis pumila* Pursh, which he shows should be restored to specific rank.—GEORGE E. OSTERHOUT (*ibid.* 506-508) has described six new forms from Colorado, under *Allium* (2), *Artemisia* (3), and *Agoseris* (1).—AVEN NELSON (from tenth annual report of the Wyoming Exper. Sta.) has published under the title "The Cryptogams of Wyoming" a list of those species which have been secured in the botanical survey of the state.—E. KOEHNE (Engler's Bot. Jahrb. 29:161-168. 1900) has completed his account of new Lythraceæ.—L. DIELS (*ibid.* 169-320. pls. 2-5) has begun the publication of the flora of Central China, a general consideration of the nature of the region covered being followed by a list of species, including new ones, from Filicales to Caryophyllaceæ inclusive. The paper contains descriptions of 9 new Ferns, 26 new Monocotyledons, and 13 new Dicotyledons so far as the group is considered. *Smilax* contains 10 new species, and a conspectus of *Fagus* is

<sup>23</sup> Ein natürliches System von Giftwirkung. 1893.

given, 7 species being recognized, one of which is new.—KARL FRITSCH (*ibid.* Beiblatt 5-23) has published an account of the Gesneriaceæ of Brazil.—OTTO V. SEEMAN (*ibid.* Beiblatt 28) has published two new species of *Salix* (*S. pseudolapponum* and *S. aemulans*) from Colorado, collected and distributed by Baker, Earle, and Tracy.—F. V. COVILLE (Proceedings of the Washington Academy of Science 2: 275-286. *pl.* 15. 1900) has given an account of the tree willows of Alaska, recognizing five species, one of which (*S. amplifolia*) is described as new.—A. W. EVANS (*ibid.* 287-314. *pls.* 16-18) has published notes on the Hepaticæ collected in Alaska by the Harriman Expedition, the list containing sixty-three species.—C. L. POLLARD (Proc. Biol. Soc. Washington 13: 169. 1900) has described a new violet from Alabama.—E. L. MORRIS (*ibid.* 171-182) has discovered in West Virginia forty-seven species heretofore unreported from the state, and also new subspecies of *Polypodium vulgare* and *Vernonia gigantea*.—JOHN M. HOLZINGER (Asa Gray Bull. 8: 95-99. *pl.* 6. 1900) has reported from Yellowstone National Park a *Polytrichum* (*P. Jensenii* Hagen) new to the United States, and heretofore known from northern Europe.—K. M. WIEGAND (Bull. Torr. Bot. Club 27: 511-527. 1900) has published upon *Juncus tenuis* and some of its North American allies, discussing thirteen species, and describing four species as new.—P. A. RYDBERG (*ibid.* 528-538), in continuing his "Studies on the Rocky mountain flora," presents the species of Melanthaceæ, finding that the family is represented in the region under consideration by at least five genera and seventeen species. Of these, one genus (*Stenanthella*) and seven species are described as new. The new genus is based on *Stenanthium occidentale* Gray, and the new species belong to *Tofieldia* (1), *Veratrum* (1), and *Zygadenus* (5).—W. N. SUKSDORF (Bot. Monats. 18: 153-156. 1900), in continuing his account of Washington plants, has described new species or varieties of *Pentstemon*, *Mimulus*, *Castilleja*, *Aphyllon*, and *Listera*.—In Mém. Herb. Boiss. (no. 20, October 1900) publication of the African flora is continued, new species being described under numerous families by the various collaborators, and under Scrophulariaceæ STAPP describes and figures a new genus under the name *Dintera*.—J. M. C.

ARNOLDI'S<sup>14</sup> third contribution to the morphology of gymnosperms deals with the embryology of *Cephalotaxus Fortunei*. Systematists have placed *Cephalotaxus* between *Phyllocladus* and *Ginkgo* on the one side and *Torreya* and *Taxus* on the other. In anatomical features it closely resembles *Taxus*, while the structure of its ovule allies it more nearly with *Ginkgo* and the cycads. It was the object of this study to determine whether embryology would throw any light upon the relationships of this interesting form. The material was obtained from a botanical garden in southern Russia. Alcohol

<sup>14</sup> ARNOLDI, W.: Beiträge zur Morphologie der Gymnospermen, III. Embryogenie von *Cephalotaxus Fortunei*. Flora 82: 46-63. *pls.* 1-3. 1900.

was used for fixing, and Zimmermann's fuchsin and iodine green method for staining.

As in many other gymnosperms, the ovules require two years for ripening. In the first year the nucellus and integument are formed and pollination takes place. In the spring of the second year the archesporium and embryo-sac are formed; endosperm appears in May, and before the end of the month the archegonia are developed; fertilization occurs about the middle of June, and during August the embryo is fully formed.

The principal results of the study are as follows:

1. The neck of the archegonium consists of two cells.
2. The material for the growth of the egg is supplied by the nuclei of the cells immediately surrounding it, and has at first the form of very small droplets; after these have passed into the egg they attain considerable size and some complexity of structure; and serve as nourishment for the embryo in its earliest stages.
3. The nucleus of the central cell divides, but does not form a ventral canal cell. The upper part of the central cell with its nucleus then becomes mucilaginous, destroys the neck cells, and presses out from the egg.
4. The pollen tube contains four nuclei, the two male cells being of the same size.
5. After the pollen tube has discharged its contents, one of the male nuclei fuses with the egg nucleus, while the other remains in the upper part of the egg where it may divide amitotically. The fusion nucleus moves to the middle of the egg and divides three or four times. The daughter nuclei divide at the bottom of the archegonium and thus there arises a number of free cells. These cells then become arranged into tiers in much the same manner as in *Taxus*, according to Jaeger's recent description. The later stages in the development were described long ago by Strasburger.—C. J. CHAMBERLAIN.

THE LIFE HISTORY of a new and interesting member of the Chytridiales has been described by Gobi.<sup>15</sup> This form, *Rhizidiomyces ichneumon*, is found on one of the Volvocaceæ, *Chloromonas globulosa*, and may multiply so rapidly as to create an epidemic among these unicellular organisms. The swarm spores of the fungus settle down on the surface of the *Chloromonas*, both the motile and resting cells, and there put forth a process which enters the host. While most of the protoplasm of the parasite remains outside of the algal cell, a connection becomes established with the host by the enlargement of the entering process into a sort of bulb on the inside, and the development from this of several filamentous outgrowths. These latter push

<sup>15</sup>Ueber einen neuen parasitischen Pilz, *Rhizidiomyces ichneumon*, nov. sp., und seinen Nährorganismus, *Chloromonas globulosa* (Perty). Scriptis Botanicis Horti Univ. Imp. Petropolitanae 15: 251-292. pls. 6-7. 1899.

into the Chloromonas cell, which eventually dies. The amount of protoplasm in the Rhizidiomyces increases rapidly, until the portion outside of the algal cell is almost as large as the host itself. This part then prepares to develop and discharge the swarm spores. The protoplasm is gathered from the interior, and a neck is formed through which the divided contents escape into the water as small uniciliate zoospores that swim slowly away.—B. M. DAVIS.

GOBI<sup>16</sup> has investigated the life history of a new species of *Pythium* that lives in the dead and dying filaments of *Vaucheria*. *Pythium tenue* is a very delicate form whose hyphae can hardly be more than  $1-3\mu$  in diameter. It grows in the interior of the *Vaucheria* filaments, sending an occasional free end of the mycelium through the wall of the alga to the exterior. The end of such a hypha becomes swollen, but is not cut off by a cross wall as is usually the case when sporangia are formed. However, the protoplasm in its interior develops several swarm spores which shortly escape. The swarm spores quickly germinate, coming to rest in the exterior of the *Vaucheria* filament, where each puts forth a germ tube that pierces the cell wall and within three hours may develop a long hypha. The sexual organs are small and chiefly interesting from the fact that the end of the antheridial filament is not cut off as a distinct cell. The oospore is  $8-9\mu$  in diameter and possesses a smooth wall. Nothing is known of its cytoplasmic structure, and its germination has not been observed.—B. M. DAVIS.

MR. O. F. COOK has published in *Science* (12:475-481. 1900) his very interesting paper on "The method of types in botanical nomenclature." After a well organized discussion of the subject, the following general statements are advanced. "In biology a species is a coherent or continuous group of organisms." "For nomenclatorial purposes a species is a group of individuals which has been designated by a scientific (preferably a Latin adjective) name, the first individual to which the name was applied constituting the type of the species." "For purposes of reference and citation specific names which appeared previous to the *Species Plantarum* of Linnaeus are not regarded in botanical nomenclature." "A genus of organisms is a species without close affinities, or a group of mutually related species." "A generic name is established in taxonomy when it has been applied to a recognizable species." "The generic taxonomy of plants may be treated as beginning with Tournefort's *Institutiones* (1700)."—J. M. C.

MR. L. A. BOODLE has begun the publication of a series of papers on the comparative anatomy of the Hymenophyllaceae, Schizaeaceae, and Gleicheniaceae, the first<sup>17</sup> dealing with the Hymenophyllaceae. A summary

<sup>16</sup> GOBI: Entwicklungsgeschichte des *Pythium tenue*, nov. sp. Scriptis Bot. Horti Univ. Imp. Petropol. 15:211. pls. 4-5. 1899.

<sup>17</sup> Annals of Botany 14:455-496. pls. 25-27. 1900.

of his results is as follows: the stem is monostelic, and one leaf trace passes to each leaf; the stele contains no pith, and is of several types, (1) a xylem mass with internal protoxylem, connected with leaf traces, (2) a xylem mass with indefinite scattered protoxylem, (3) a xylem mass with peripheral protoxylem, (4) sub-collateral, (5) a collateral bundle. The author thinks that the sub-collateral structure has probably been derived from a more complicated type by reduction, and that the filmy habit of *Hymenophyllum* is probably not primitive.—J. M. C.

MR. R. F. SHOVE has published<sup>18</sup> an account of the structure of the stem of *Angiopteris evecta*, which in 1864 had been described in detail by Mettenius, but from whom the present author differs in several particulars. The position of the protophloem in the stem stele is said to be anomalous, occurring on the outer side of the stele in the form of a discontinuous arc. The centrifugal growth of the phloem is also said to be contrary to that described for most other ferns, but the other Marattiaceæ are not included in this comparison. The mesarch and endarch structure of the steles was confirmed, and also the presence of several initials at the apex of the stem. — J. M. C.

W. L. BRAY discusses the relations of the North American flora to that of South America.<sup>19</sup> Few new conclusions are reached, but there is an interesting summary of the known data on the subject. The most important agencies which have brought about relationships are those which have acted in the past; the endemic northern floras of Mexico and the Andes are to be referred to such causes. Minor agencies of distribution are still active, especially those along the gulf zone and those due to man.—H. C. COWLES.

ULE DESCRIBES several isolated observations<sup>20</sup> that he has made concerning the influence of animals upon plants in the tropics. These observations have to do mainly with pollination and seed dispersal. He opposes the view that palms and other plants which develop a considerable amount of heat in the flowers do so with any relation to pollination. This theory is *a priori* improbable, and the author adds that no protection would be necessary to the insects in tropical climates, and furthermore that the insects do not fly at night.—H. C. COWLES.

MISS ETHEL N. THOMAS has announced<sup>21</sup> the discovery of double fertilization in *Caltha palustris*. The original discoveries of this phenomenon, it will be remembered, were among the Liliaceæ, so that the discovery of

<sup>18</sup> Annals of Botany 14: 497-525. pls. 28-29. 1900.

<sup>19</sup> Science 12: 709-716. 1900.

<sup>20</sup> Ber. deutsch. Bot. Ges. 18: 122-130. 1900.

<sup>21</sup> Annals of Botany 14: 527-535. pl. 30. 1900.

Miss Thomas was interesting in extending the phenomenon to the dicotyledons. Before the publication of her paper, however, Nawaschin had announced his discovery of double fertilization in *Helianthus* and *Rudbeckia*, and its probable occurrence in *Delphinium*; while more recently Land<sup>22</sup> has included *Erigeron* and *Silphium*.—J. M. C.

W. H. LONG, JR.,<sup>23</sup> gives an interesting preliminary account of the ecological distribution of fungi in the vicinity of Austin, Texas. In general the conditions do not seem to favor a great abundance of fungi, the numbers being limited by the nature of the soil, the paucity of forests, and the climate. Parasitic forms are limited by reason of the xerophytic nature of the host leaves. The saprophytic forms are ecologically subdivided into dung inhabiting species, cedar brake fungi, post oak land species, species of grassy places, epixylous species, and species of open rocky soil.—H. C. COWLES.

MR. JAMES BRITTEN has done good service to taxonomists in publishing in the *Journal of Botany* (38: 430-443. 1900) a textual reproduction of the two pages of Linnaeus's *Systema Naturae*, ed. i, which give in tabular form the classification of plants. The occasion for this is the great rarity of the work, which is a folio, whose preface is dated from Leyden, July 23, 1735.—J. M. C.

DR. HERMANN VON SCHRENK has published an account<sup>24</sup> of two diseases of red cedar, one called "white rot," and caused by *Polyporus juniperinus*, described as a new species; the other called "red rot" or "pecky cedar," and caused by *Polyporus carneus*. Seven plates, mostly from photographs of diseased wood, accompany the paper.—J. M. C.

THE CALYX HYDATHODES which have been found by Koorders are confined to tropical plants. Shibata<sup>25</sup> adds five Japan species, and finds that the hydathodes secrete mainly water, but that they also secrete some sugar and thus have a relationship to nectaries.—H. C. COWLES.

<sup>22</sup> Botanical Gazette 30: 252-260. 1900.

<sup>23</sup> Bull. Torr. Bot. Club 27: 579-588. 1900.

<sup>24</sup> Bulletin 21, U. S. Dept. of Agric. Div. of Veg. Phys. and Path. 1900.

<sup>25</sup> Bot. Centralbl. 83: 350. 1900.

## NEWS.

DR. DAVID GRIFFITHS has been appointed professor of botany in the University of Arizona, and also botanist to the Experiment Station.

DR. E. PALLA, *privat-docent* in the University of Graz, left home at the end of September for a long anticipated sojourn in Buitenzorg, Java.

DR. M. VON RACIBORSKI has been appointed professor of botany and director of the botanical gardens in the agricultural school at Dublaney, near Lemberg.

A BOTANICAL SCHOOL is being erected at a cost of \$20,000 in Schenley Park, Pittsburgh, being intended especially for teachers and classes from the public schools.

PROFESSOR L. M. UNDERWOOD spent the summer in studying American ferns in the British Museum, in the Kew Gardens, and in the Cosson Herbarium in Paris.

DR. N. L. BRITTON represented the New York Botanical Garden at the recent International Botanical Congress at Paris, and was also an accredited delegate from the U. S. Government.

OF THE THIRTY names selected to represent America's greatest men in the Hall of Fame of the University of New York only two are those of naturalists. One of these is Asa Gray and the other J. J. Audubon.

WE LEARN from *Science* that the Yale Forestry School has opened with an enrollment of seven regular students and seventeen from other departments, the residence of the late Professor O. C. Marsh being used as a school building. The degree of Master in Forestry will be given to such students of the school as have previously received the bachelor's degree from collegiate institutions of high standing.

MUHLENBERGIA is the title of a new journal of botany, whose initial number is dated November 10, and whose editor and publisher is Mr. A. A. Heller. The publication office is Lancaster, Pa., and the subscription price is \$1.00 a volume, the volume to contain not less than 150 pages. The journal is to be issued at irregular intervals, and the first number (8 pp.) consists of a list of changes in nomenclature by the editor.

THE *Journal* of the New York Botanical Garden is designed to be chiefly an organ of communication with the members of the Garden organization (now numbering about a thousand), who are thus to be kept informed of



the progress and development of the garden in all its departments and of the activities of people connected with it in any way. At present technical articles will not be published, though botanical notes, news, and reviews will often be included.

THE *O. S. U. Naturalist* is the title of a new journal whose first number appeared in November. It is a journal devoted "more especially to the natural history of Ohio," and is the official organ of the Biological Club of the Ohio State University. It is published monthly during the academic year, and its subscription price is fifty cents. The editor-in-chief is Professor John H. Schaffner, of the University. The first number contains two botanical papers by Professor W. A. Kellerman.

IN THE *Bulletin* of the Torrey Botanical Club for October, some of the papers presented at the celebration of "Torrey Day" in connection with the last meeting of the A. A. A. S. are published. Dr. N. L. Britton has written an exceedingly interesting sketch of Dr. Torrey as a botanist, and follows it by a bibliography; Dr. Edward S. Burgess has presented the work of the Torrey Botanical Club; while Dr. James Hyatt, one of the earliest members of the club, Professor T. C. Porter, and Professor Charles H. Peck, have put in permanent form some delightful personal reminiscences of Dr. Torrey.

BY THE WILL of the late Charles H. Smith, of Providence, R. I., the botanical department of Brown University receives \$2,000 the income from which is to be expended under the approval of the senior professor of botany, now Professor W. W. Bailey. The city of Providence is made residuary legatee for the development of parks. The income, which is likely to amount to ten or twelve thousand dollars annually, is to be applied to the purchase of plants for the parks, greenhouses, etc., under the advice and direction of the superintendent of parks, and the senior professor of botany of Brown University. One thousand dollars is bequeathed to the city as a trust fund, the income to be used for purchasing microscopes for the high schools.

MR. B. T. GALLOWAY has been appointed Director of Plant Industry, Mr. Albert F. Woods has been promoted to be Chief and Mr. M. B. Waite Assistant Chief of the Division of Vegetable Physiology and Pathology, U. S. Department of Agriculture. The Secretary has issued the following general order: "For the purpose of unifying the work of certain branches of the department, it is hereby ordered that the Chief of the Division of Vegetable Physiology and Pathology, the Chief of the Division of Agrostology, and the Chief of the Division of Pomology confer upon all matters of general policy and plan with the Superintendent of Experimental Gardens and Grounds, who is hereby designated as Director of Plant Industry. In carrying out this order the several branches of the department named will maintain their integrity and organization."

NOW THAT the *Pflanzenfamilien* is about complete, the energetic editor, Dr. Adolph Engler, has entered upon an even more gigantic undertaking. The publisher, William Engelmann, announces the beginning of a work, *Das Pflanzenreich*, which is to be a conspectus of the plant kingdom, *i. e.*, a critical enumeration of all orders, families, genera, sub-genera, species, and varieties. It is proposed to issue the work in separately paged parts, each family being complete in itself, with its own index, though smaller families will be united in parts containing two to four signatures. The arrangement of the matter will be as follows. At the head of each family will be given its literature, including both systematic and general works. Purely systematic monographs of genera, however, will be listed under the appropriate genus. The general sections — vegetative organs, anatomical relations, etc. — will be written in German and briefer than in the *Pflanzenfamilien*; the diagnoses of families, genera, sections, and the enumeration of species will be in Latin. Only important synonyms (with the year), and only really good characteristic illustrations will be cited. Exsiccatae may also be cited in doubtful cases. The style of citation will be uniform. Thirty collaborators have agreed upon the nomenclature to be used. It must not be supposed that this is merely a revision of the *Pflanzenfamilien*. That work will be kept up to date by biennial supplements, and the new one, beginning with the families most in need of new elaboration will proceed on its own lines. Twenty years, it is anticipated, will be required for its completion. The support, scientific and financial, of the Prussian *Kultusministerium* and the Prussian Academy of Sciences, together with the proved executive ability of editor and publisher, are strong guarantees that the work will progress to a satisfactory completion.

## GENERAL INDEX.

The most important classified entries will be found under Contributors, Personals, and Reviews. New names and names of new genera, species, and varieties, are printed in **bold-face type**; synonyms in *italics*.

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